December 2019

Diamond Zoysiagrass Response and Seedhead Control Using Plant Growth Regulators

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DIAMOND ZOYSIAGRASS RESPONSE AND SEEDHEAD CONTROL USING PLANT GROWTH REGULATORS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Plant and Environmental Science

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by
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December 2019

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ABSTRACT

Golfers have steadily increased demands for putting green quality, including distance and smoothness. ‘Diamond’ zoysiagrass has emerged as a viable C_4 turfgrass to be used by golf courses as putting greens or fairways and tees. ‘Diamond’ zoysiagrass exhibits multiple benefits over other C_4 and C_3 turfgrasses, but an undesirable trait is seedhead production.

Three studies were conducted at Clemson University from April 2018 to November 2019 to investigate the effects of plant growth regulators (PGRs) on ‘Diamond’ zoysiagrass’ turf quality (TQ), lateral recovery and seedhead production. Study 1 tested PGRs in a field setting on the TQ, seedhead production and root mass of a ‘Diamond’ zoysiagrass putting green. Study 2 tested PGRs on the TQ, seedhead production, clipping and root mass of ‘Diamond’ zoysiagrass in a greenhouse setting. Study 3 tested PGRs effects on the lateral growth of ‘Diamond’ zoysiagrass.

All three studies included treatments of trinexapac-ethyl (TE), prohexadione-calcium (PC), paclobutrazol (PB), flurprimidol (FL), ethephon (EP) and simazine (SI). The first study determined initial seedhead production was best predicted by 12.4 h photoperiod but accumulated growing degree days (GDD) was also fairly accurate. Compared to untreated, seedhead count was reduced through treatments SI and SI + TE by 80 and 75%, respectively, during fall seasons. Treatments did not consistently increase TQ above untreated. In fall 2018 at 14 days after treatment (DAT), SI and SI + TE decreased TQ to 5.5 and 5.3, respectively, significantly below the untreated’s rating of 7.

However, in fall 2019 at 14DAT, SI and SI + TE were similar to control. Normalized difference of vegetative index (NDVI) reflected similar trends of TQ.

Study 2 illustrated 4 applications of TE, SI, PC, FL and SI on ‘Diamond’ zoysiagrass improved TQ to 7.9, 7.9, 7.8, and 7.8, respectively, all significantly greater than untreated’s 6.9. At this date EP reduced TQ to 5.6, significantly below untreated. Seedhead count after 5 applications was decreased by SI by 63% compared to the untreated, consistent with study 1. Interestingly, TE also reduced seedhead count by 41% below untreated, while EP increased count by 68% above untreated. Study 3 revealed at 16 weeks after initial treatment (WAIT) lateral recovery was decreased by FL, TE, PC, and PB by 20, 14, 12, and 11%, respectively, from untreated.

DEDICATION

I dedicate this work to Mr. Gerald Harris, a family man who showed me how to work hard and enjoy life's blessings. I love you granddaddy and can't wait to see you again.

ACKNOWLEDGMENTS

My major professor Dr. Bert McCarty deserves the first and most gracious acknowledgement. He gave me opportunities to learn from his experiences and knowledge, and I will be forever thankful for his patience with a bulldawg. I consider myself extremely blessed to have worked under such an icon in the turfgrass management industry. I would like to thank him for funding my assistantship and research.

I also greatly appreciate Dr. Billy Bridges and Dr. Matthew Cutulle for serving on my graduate committee. Dr. Bridges provided ample help in analyzing data and greatly contributed to this project's success. Dr. Cutulle gave me opportunities to learn outside the turfgrass management field.

I am grateful for the colleagues I had during my time at Clemson. I would especially like to thank Nate Gambrell for his help setting up trials and his advice. I would also like to thank Philip Brown, Josh Weaver, Bobby Kerr, Jacob Taylor, and Tee Stoudemayer for camaraderie and direction.

I also want to thank my entire family for their endless love and support of me. Thank you for shaping my life for the better, I can only hope to repay the favor someday.

To Daly, thank you so much for sharing burdens with me. I will always remember how your selfless love inspired me. You make me so proud.

Finally, I thank my lord and savior Jesus Christ. Without his grace on the cross I would still be lost and without purpose.

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CHAPTER ONE

INTRODUCTION

Putting Greens

Golf course putting greens are intensively managed turfgrass systems with delicate agronomic challenges. Golfers' demands for increased putting distances or "speed" lead to low mowing heights, plant growth regulator use, regular sand topdressing and lightweight rolling to increase ball roll distance, and smoothness (Fagerness and Yelverton, 2001; Hartwiger et al., 2001; McCarty et al., 2011; McCullough et al., 2005b). Putting greens until 1960 were constructed with native soils or mixtures of native soil, sand and organic matter in a 1:1:1 ratio before the United States Golf Association (USGA) released methods for putting green construction (Ferguson, 1965; Holmes, 1967). This rootzone of a precise mixture of textured sands and organic matter over pea gravel and drain tiles creates a perched water table (Ferguson, 1965; USGA 2018). Benefits of this method include rapid drainage, minimal soil compaction, nutritional consistencies, water management predictability and avoidance of native soil pests (Hummel, 1993; O'Brien and Oatis, 2018; USGA, 2018).

Zoysiagrass

Zoysiagrass (*Zoysia* spp.) is a warm season or C_4 turfgrass utilized on golf courses, home lawns, sports fields and commercial landscapes throughout the transition zone (McCarty, 2018). Common zoysiagrass species are *Zoysia japonica* and *Z. matrella*

(Patton et al., 2017), but *Zoysia tenuifolia*, *Z. pacifica*, *Z. minima* have attractive qualities used to breed new varieties (Chandra et al., 2017; Engelke and Anderson, 2003; Kimball et al., 2013). *Zoysia* spp. have a larger adaptable range than other C_4 grasses due to excellent tolerances of shade, freezing temperatures and photosynthetically active tissue at low temperatures (Engelke and Qian, 2000; Hinton et al., 2012; Rogers et al., 1977; Sladek et al., 2009). *Zoysia* spp. exhibit slower lateral growth rates compared to other C_4 grasses, but typically develop dense thatch layers (Dunn et al., 1981; Fry and Dernoeden, 1987; McCarty, 2018).

Z. matrella or 'Manilagrass', was introduced to the United States by Charles V. Piper, who originally collected it in 1912 from a Philippine Islands' seashore environment (USDA Bureau of Plant Industry, 1915; Patton et al., 2017). 'Diamond' zoysiagrass (*Zoysia matrella* (L.) Merr.) was originally collected by Dr. Milton C. Engelke in 1982 from Asia (Murray and Engelke, 1983). 'Diamond' zoysiagrass was evaluated at Texas A&M Agrilife Research and Extension center in Dallas, Texas, and was patent registered in 2002 (Engelke, 1998; Engelke et al., 2002).

'Diamond' zoysiagrass utilizes stoloniferous and rhizomatous growth with extremely high tiller density (Trappe et al., 2011), a desirable trait for sod production (Engelke et al., 2002; Lulli et al., 2011), and preventing weed species colonization. 'Diamond' zoysiagrass has a very fine leaf texture and tolerates mowing heights less than 2.5 mm (Menchyk et al., 2012), making it suitable for golf course putting greens (Patton et al., 2017).

Patton et al. (2004) reported *Zoysia* spp. establishment from seed is difficult, as

‘Zenith’ (*Zoysia japonica* Steud.) was significantly slower than ‘Mirage’ bermudagrass (*Cynodon dactylon* var. dactylon (L.) Pers.). ‘Diamond’ has the slowest lateral growth rate among multiple *Zoysia* spp. (Fry and Dernoeden, 1987; Sladek et al., 2011), a disadvantage in establishment and lateral recovery. Sprigging a Diamond green took a minimum of 12 wk to reach 100% coverage (Stiglbauer et al., 2009), while sprigged ‘Miniverde’ bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy) took as few as 7 wk (Briscoe et al., 2012). Multiples studies indicate nitrogen application rates to not significantly influence *Zoysia* spp. establishment from sprigs (Richardson and Boyd, 2001; Stiglbauer et al., 2009) or seed (Patton et al., 2004). Establishing Diamond greens with sod provides playability in approximately 3 wk but at an increased cost (Donald Garrett, personal communication, 2018).

Zoysia spp. are noted for increased shade tolerance compared to other C_4 grasses (Ervin et al., 2002; Bunnel et al., 2005; Qian and Engelke, 2000; Sladek et al., 2009), but this varies between *Zoysia* spp. and cultivars (Trappe et al., 2011; Wherley et al., 2011). ‘Diamond’ zoysiagrass’ shade tolerance is superior to all currently available *Zoysia* spp. and cultivars, maintaining high turfgrass quality under continuous 60% shade (Engelke et al., 2002; Qian and Engelke, 2000; Sladek et al., 2009; Wherley et al., 2011; Atkinson et al., 2012). In two separate studies ‘Diamond’ zoysiagrass grown in >50% shade treated with trinexapac-ethyl (TE) had similar turfgrass quality to 0% shade (Atkinson et al., 2012; Qian et al., 1998). Qian et al. (1998) also reported total nonstructural carbohydrate levels in ‘Diamond’ zoysiagrass under shade increased when treated with TE, improving winter survival (Qian and Engelke, 2000).

Zoysia spp. is less drought tolerant than *Cynodon* spp. (Wherley et al., 2014; Patton, 2017). This is due, in part, to *Zoysia* spp. having shallower rooting systems than *Cynodon* spp. (Fuentealba et al., 2015; Huang et al., 1997; Marcum et al., 1995), a slower rate of root development (Zhang et al., 2015), and higher evapotranspiration rates compared to other C_4 grasses (Carrow, 1995). Under drought stress, 'Meyer' zoysiagrass displayed distinct leaf rolling characteristics to avoid water loss, while other *Zoysia* spp. accumulated solutes in leaves to maintain turgor (Qian and Fry, 1997). Specifically, 'Diamond' zoysiagrass required the greatest amount of supplemental irrigation and had slower regrowth rates following a drought event (Wherley et al., 2014; White et al., 2001).

Zoysia matrella was originally collected from seashores of Philippine Islands under high salinity pressure, and possesses specialized salt secretion glands (Patton et al., 2017; Marcum et al., 1998). *Zoysia matrella* compared to other *Zoysia* spp. had higher gland density and lower rates of leaf firing when treated with NaCl solution (Marcum et al., 1998). 'Diamond' zoysiagrass repeatedly exhibited the highest salinity tolerance of multiple *Zoysia* spp. and cultivars (Engelke, 1998; Engelke et al., 2002; Qian et al., 2000). Salt tolerance of turfgrass systems is desirable as recycled water use increases (Gelernter et al., 2015).

Finer textured *Z. matrella*, *Z. pacifica* and *Z. minima* have proven to be more sensitive to freezing than coarser textured *Z. japonica* (Dunn et al., 1999; Forbes, 1952; Hinton et al., 2012; Okeyo et al., 2011; Patton and Reicher, 2007). 'Diamond' zoysiagrass was least tolerant to freezing in a study with 34 other *Zoysia* spp. and cultivars (Patton and Reicher, 2007), warranting use of covers for putting greens in the transition zone (Anderson et al., 2002; Goatley et al., 2005). Experimental hybrids of finer textured *Zoysia* spp. with coarser *Z. japonica* have proven to have desirable freezing tolerance (Chandra et al., 2017; Fry et al. 2017). *Zoysia* spp. possess marginally better freezing tolerance over bermudagrass cultivars (Anderson et al., 2002; Hinton et al., 2012; Patton and Reicher, 2007). Compared to *Cynodon* spp., *Zoysia* spp. were up to 8 times more photosynthetically active at temperatures where only 12% green color tissue remained (Rogers et al., 1977), a desirable trait to tolerate cooler climates.

Zoysia spp. is often mistaken to possess low traffic tolerance but has a similar traffic tolerance to *Cynodon* spp. (Trappe et al., 2011). Finer textured *Zoysia* spp. recovery time from divots were slowest (Karcher et al., 2005), consistent with previous lateral growth rate studies (Fry and Dernoeden, 1987; Sladek et al., 2009). Trappe et al. (2011) reported 'Diamond' zoysiagrass had lower divot severity than most cultivars, requiring the most lateral force to shear. The dense canopy of 'Diamond' zoysiagrass and high lignin content (Lulli et al., 2011) likely prevent traffic damage.

Zoysia spp. maintains acceptable turfgrass quality with reduced nitrogen fertility (Schwartz et al., 2018). Dunn et al. (1995) and Soper et al. (1988) noted 98 kg N ha^{-1} yr^{-1} to be sufficient for *Zoysia* spp., but in climates with longer growing seasons, 171 kg N ha^{-1} yr^{-1} was needed to provide acceptable quality (Schwartz et al., 2018). Ball roll distance and surface firmness of 'Diamond' zoysiagrass putting greens were consistently reduced when nitrogen rates exceeded 73.5 kg N ha^{-1} yr^{-1} (Menchyk et al., 2014). *Zoysia* spp. responds more positively to urea nitrogen sources vs. nitrate, resulting in increased root and stem mass (Patton et al., 2009). Excessive nitrogen, >195 kg N ha^{-1} yr^{-1}, leads to increased thatch accumulation, often resulting in scalping (Schwartz et al., 2018; Soper et al., 1988), and increased weed pressure (Doroh and McElroy, 2010).

Recently, ‘off-types’ have become an issue in ultradwarf bermudagrass putting greens (Lowe and Foy, 2012; Reasor, 2017). ‘Off-types’ are believed to be a result of chance mutation resulting in aneuploidy. However, encroachment, mechanical spread of species and insufficient control of previous species may also be categorized as ‘off-types’ (McCarty, 2018). Reasor (2017) reported ‘off-type’ species responded differently to nitrogen fertility and plant growth regulators, making maintenance increasingly difficult. *Zoysia* spp. has relatively low threat of ‘off-type’ mutation due to possessing 20 chromosomes (Forbes, 1952; Kimball et al., 2013). Ultradwarf bermudagrass is a triploid with 27 chromosomes and is much more likely to undergo chance mutation (O’Brien, 2012).

‘Diamond’ zoysiagrass, similar to most *Zoysia* spp., is susceptible to the fungal pathogen ‘Large patch’ (*Rhizoctonia solani*) (Green et al., 1994), typically infecting plants in fall when soil temperatures are still above 10°C. This disease develops a large necrotic ring, is exacerbated by low mowing heights, excessive nitrogen, dense thatch and wet conditions (Green et al., 1994; McCarty, 2018; Obasa et al., 2012). *Zoysia* spp. have proven to be susceptible to ‘Rust’ (*Puccinia* spp.), and severe infestations will cause turfgrass thinning and necrosis (Obasa and Kennelly, 2010).

‘Diamond’ zoysiagrass compared to other *Zoysia* spp., is highly resistant to hunting billbugs (*Sphenophorus venatus vestitus* Chittenden), a common turfgrass insect pest in southern states (Reinert et al., 2011). Crosses of *Z. japonica* × *Z. matrella* were less susceptible to bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) than 'Meyer' zoysiagrass (Fry and Cloyd, 2011). 'Diamond' zoysiagrass is moderately resistant to fall armyworm (*Spodoptera frugiperda*), and tawny mole cricket (*Neoscapteriscus vicinus*) (Braman et al., 2000). However, 'Diamond' zoysiagrass is highly susceptible to *Eriophyes zoysiae* (zoysiagrass mite), introduced to the United States in 1982 (Baker et al., 1986).

Turfgrass Seedhead Production

Turfgrass inflorescences or seedheads are reproductive organs for grasses. Seedheads are only present when the plant enters a reproductive stage after maturity (McCarty, 2018; Taiz and Zeigler, 2010). Turfgrass seedheads form elongated stems from the crown, and a flowering culm forms on top of this stem. An inflorescence is divided into spikelets, composed of two reduced leaves called glumes enclosing one or more florets. Florets consist of a lemma, palea and an enclosed flower, and mature florets are referred to as 'grass seed' (McCarty, 2018).

Inflorescence formation occurs in four phases. First is maturation of the plant because immature plants will not respond to environmental conditions typically encouraging inflorescence formation. Second is induction of flowering stimulus caused by either vernalization or photoperiodic induction. Third is a transition of the stem apex from a vegetative to a flowering axis. Fourth is formation of branches, spikelets and florets, and inflorescences rising above leaves (Turgeon, 2012).

Vernalization is a low temperature required by some plants to initiate flowering. A range of 0-10°C is required for some C_3 grasses to initiate flowering, while higher night temperatures of 12-18°C will delay their flowering. C_4 plants only initiate flowering when night temperatures are above 12-16°C (Turgeon, 2012; McCarty, 2018).

Photoperiod or day length is a primary factor for some plants to initiate flowering. Plants depend on phytochrome, a protein kinase within leaves, to signal photoperiod. Red light converts an inactive form of phytochrome to an active form, and far-red light converts an active form back to an inactive form. Phytochrome drives responses including 'florigen' protein formation in leaves, which transports to apical meristems to induce flowering (Taiz and Zeiger, 2010).

C_3 turfgrasses are typically long-day plants (LDP), which tend to flower in late spring to summer when nights become shorter than a critical duration of darkness. C_4 turfgrasses are typically short-day plants (SDP), which tend to flower in late summer to fall when nights exceed a critical duration of darkness. Day-neutral plants do not require specific day lengths and will flower as soon as they are mature. Annual bluegrass (*Poa annua* L.) and *Cynodon* spp. are believed to be day-neutral (Johnson and White, 1997; McCarty, 2018).

Orientation of spikelets and rachillas differ among turfgrasses. Spikelets may lack individual stalks and attach directly to the main axis in spike inflorescences (McCarty, 2018). Wheat (*Tritichum* spp.) and barley (*Hordeum* spp.) form spike inflorescences. Spikelets with individual unbranched stalks from the main axis are raceme inflorescences. St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntza), zoysiagrass, bahiagrass (*Paspalum notatum* Flugge) and centipedegrass (*Eremochloa ophiuroides* (Munro) Hack) form a raceme inflorescence. Spikelets may branch from individual stalks attached to the main axis to create panicle inflorescences. Bluegrasses (*Poa* spp.), creeping bentgrass (*Agrostis stoloniferia* L.) and tall fescue (*Lolium arundinacea* (Schreb.) Darbysh.) form panicle inflorescences (McCarty, 2018).

Stamens, male portions of flowers, typically are formed of pollen bearing anthers and supporting filaments. Female portions are termed pistil, typically formed of a single ovary and two stigmas and styles. Transfer of pollen from anthers to stigmas is termed 'pollination'. Most grasses self-pollinate, but cross-pollination allows transfer of genetic material (Kimball et al., 2013). Once pollen contacts stigmas, a pollen tube grows through styles and into ovules within ovaries, and two sperm nuclei are released. A one sperm nucleus conjoins with an egg to create a zygote, and the other conjoins with two polar nuclei to form an endosperm cell, termed 'double fertilization'. Zygotes form embryos, and endosperm cells develop into endosperms becoming food sources for embryos during germination. Kentucky bluegrass is able to perform apomixis, an asexual reproduction process where male and female gametes do not fuse. These seeds will have an identical genetic makeup to female parents, and true-to-type seed can be produced (Turgeon, 2012).

Zoysia matrella produces seedheads, similar to other *Zoysia* spp. (Forbes, 1952; McCarty, 2018; Patton et al., 2018; Youngner, 1961). *Zoysia* spp. form aesthetically unpleasing seedheads in both spring and fall (McCullough et al., 2017), while 'Meyer' typically only forms seedheads in spring (Daniels and Nicoludis, 2019; Patton et al., 2018).

Two separate greenhouse experiments indicated a minimum of 27°C resulted in maximum seedhead production in *Zoysia* spp., including *Z. matrella* (Forbes, 1952; Youngner, 1961). These experiments also indicated photoperiods between 8 and 10 h triggered seedhead production, while 12 h photoperiods yielded no seedhead formation. Nakamae and Nakamura (1984) reported reductions in light and nitrogen lead to increased seedhead production. Yeam et al. (1984) determined a minimum of twelve nodes in the upright culm was required before *Z. matrella* would flower.

McCullough et al. (2017) recorded 'Diamond' zoysiagrass' initial seedhead production in field using photoperiod and accumulated growing degree days (GDD) (base 10°C) for the calendar year to determine emergence factors. Initial seedhead production ranged from 167 to 196 GDD and 1949 to 2230 GDD in spring and fall, respectively. Photoperiod ranged from 12.7 to 12.9 h in both spring and fall (McCullough et al., 2017). Over two years, GDD was determined to be a more accurate predictor of initial seedhead formation in spring, but photoperiod was a more accurate predictor in fall (McCullough et al., 2017). Similar GDD methods have been applied to annual bluegrass to predict seedhead formation (Calhoun, 2010). Timing is crucial for cost effectiveness, planning and duration, as applied products must be absorbed by turfgrass before seedhead inhibition to be effective (McCarty, 2018).

'Diamond' zoysiagrass' ball roll distance is typically less than ultradwarf bermudagrass greens (Donald Garrett, personal communication, 2018; Menchyk et al., 2014), likely due to resistance from upright growth and high lignin content in *Z. matrella* leaf tissue (Lulli et al., 2011). Ball roll distance and smoothness of putting greens are also reduced by presence of annual bluegrass seedheads (Gelernter and Stowell, 2001; Kane and Miller, 2003).

Seedhead production consumes carbohydrates produced by turfgrass through photosynthesis. Loss of finite carbohydrates and energy molecules is detrimental to other plant processes (Taiz and Zeigler, 2010). Preventing seedhead formation with PGRs would conserve photosynthetic resources for plant growth and storage. Storage of carbohydrates in stolons and rhizomes is considered the primary factor in winter survival of C_4 turfgrasses (Dunn and Nelson, 1974; Qian and Engelke, 2000; Rogers et al., 1977). Seedhead presence may also accelerate decline of mower reel and bedknife sharpness (Donald Garrett, personal communication, 2018), increasing shearing of leaves, and reducing turfgrass quality.

Ethylene

Ethylene was discovered in the late 19th century when coal streetlamps under tree branches resulted in decreased stem elongation, twisting of plants and thickening of stems, termed ‘triple-response’. In 1901, Russian scientist Dimitry Neljubow proved the active component of streetlamp gas was ethylene. In 1910, H. H. Cousins discovered ethylene was synthesized in plants, and stimulated fruit ripening. R. Gane and others in 1934 identified ethylene as a natural product of plant metabolism, leading to its classification as a hormone. Ethylene inhibits growth of plant tissues, theoretically counteracting abscisic acid and gibberellic acid (Taiz and Zeigler, 2010). Ethylene is also inhibits lateral bud development, root growth, and cell elongation; It slows cell differentiation, promotes leaf abscission, influences some plant defense responses, and is responsible for flower induction in some plants (Davies, 2010).

Ethylene is synthesized in all higher plants from methionine in most tissues, especially during times of stress. Adenosine triphosphate and methionine result in S-adenosyl methionine (SAM) and a loss of three phosphates. SAM then produces 1-aminocyclopropane-1-carboxylic acid (ACC) via the ACC-synthase enzyme. ACC combined with oxygen and the ACC-oxidase enzyme produces ethylene (Taiz and Zeiger, 2010). Some PGRs such as ethephon (EP) stimulate ethylene production in plant to slow growth or reduce seedhead production (McCarty, 2018).

Gibberellic Acid

Gibberellic acids (GAs) were first discovered by Japanese scientists in rice plants in the 1930s via the pathogenic fungus, *Gibberella fujikuroi*. This disease caused rice plants to grow excessively tall leading to lodging and reduced seed production. By culturing the fungi, scientists were able to obtain impure crystals, a mix of three gibberellins, with plant growth-promoting activity. In the 1950s, scientists separated and characterized three different gibberellins, naming them gibberellin A1, gibberellin A2, and gibberellin A3. Gibberellin A3 was determined to be the general component in plants and could be commercially produced for applications (Taiz and Zeigler, 2010).

GAs primarily incite cell division and elongation in plants. GAs are also responsible for induction of seed germination, production of enzymes during seed germination, and induces fruit setting and growth (Davies, 2010). GAs also help break dormancy, promote early flower development, inducing flowering under non-typical flowering conditions and combine with other factors in plants to express floral regulating genes (Taiz and Zeiger, 2010). In darnel ryegrass (*Lolium temulentum* L.), GAs concentration increased under specific light conditions to promote flowering (King et al., 2006). A practical use of GA in turfgrass putting greens is to apply small amounts to heat-stressed creeping bentgrass to encourage growth, or to bermudagrass putting greens that have experienced a light fall frost to maintain desirable color for a short time (McCarty, 2018).

GAs are formed from carbohydrates created from photosynthesis following glycolysis. The process can be broken down into three stages. Stage one takes place in plastids, and glycolysis produces phosphoenol pyruvic acid and acetyl-CoA. Mevalonic acid is formed from acetyl-CoA and is the starting compound for all terpenoid biosynthesis. Mevalonic acid, a 6-carbon compound, is decarboxylated to form the first isoprenoid, a 5-carbon compound, in the pathway. Four isoprene units combine to form geranylgeranyl diphosphate, a 20-carbon linear molecule, and is converted into ent-karurene, a 6-carbon ring structure (Taiz and Zeiger, 2010).

Stage two takes place in plastid envelopes and endoplasmic reticulums. One of ent-kaurene's methyl groups is oxidized to carboxylic acid, then one of the 6-carbon rings contracts to become a 5-carbon ring, producing GA12-aldehyde. This is the first gibberellic acid formed, and the biosynthesis pathway up to GA12 is typical of plant species studied thus far (Taiz and Zeiger, 2010).

Stage three takes place in cytosols, where GA12-aldehyde is converted into biologically active 19-carbon GAs: GA4 or GA1. Three important enzymes in this stage are grouped and labeled dioxygenases and utilize 2-oxoglutarate as a co-substrate and Fe^{+2} as a co-factor (Taiz and Zeiger, 2010).

Multiple plant growth regulators (PGRs) target gibberellic acid biosynthesis to slow plant growth. These chemistries differ in their site and therefore timing of inhibition in the pathway. Plant uptake mechanisms, duration in plant tissues and soil half-lives of these growth regulators also vary (Rademacher, 2000).

Turfgrass Plant Growth Regulators (PGRs)

PGRs are defined as any compound, synthetic or natural, that alters plant growth or development; including hormones, herbicides, growth inhibitors and biostimulants (Kreuser, 2015). PGRs are commonly used by turfgrass managers to slow undesirable vertical growth of turfgrass to reduce mowing frequency and clipping volume (Fagerness and Yelverton, 2000; Johnson, 1990). PGRs are used to improve turfgrass health (Atkinson et al., 2012), density (Fagerness et al., 2002), rooting (McCullough et al. 2006; McCarty et al., 2011), color (Fagerness and Yelverton, 2000), reduce nitrogen requirements (Kreuser and Soldat, 2012), and suppress seedheads (Askew, 2017; Brosnan et al., 2012; Haguewood et al., 2013; Johnson and Murphy, 1995; Johnson and Murphy, 1996; Kane and Miller, 2003; McCullough et al., 2011; Patton et al., 2018; Woosley et al., 2003).

Class A and B PGRs are gibberellin biosynthesis inhibitors (Kreuser et al., 2015).

Class A inhibitors include trinexapac-ethyl (TE) and prohexadione-calcium (PC) and inhibit gibberellin synthesis later in the biosynthesis pathway. These chemistries are acylcyclohexanediones and structurally similar to aforementioned 2-oxoglutaric acid, allowing these PGRs to block GA metabolism. The primary target is 3beta, needed for the conversion of inactive GA20 to highly plant active GA1 (Evans et al., 1999; Rademacher, 2000). This inhibition of GA1 formation results in an accumulation of GA20, causing rapid growth suppression (Kreuser and Soldat, 2011; Rademacher, 2000; Reasor et al., 2018).

TE and PC are absorbed primarily through leaf tissue and crowns of turfgrass plants, with 80% absorption occurring within one hour, and compounds are readily transported throughout plants (Fagerness and Penner, 1998b; Rademacher, 2000). TE and PC half-lives are several hours in soil, and multiple weeks in plants, depending on air temperature and growth habits (Beasley et al., 2005; Evans et al., 1999; Kreuser and Soldat, 2011; Rademacher, 2000; Reasor et al., 2018).

Class B inhibitors such as paclobutrazol (PB) and flurprimidol (FL) inhibit gibberellin biosynthesis earlier in the formation steps than class A inhibitors. FL is a pyrimidine compound and PB is a triazole-type compound. Both chemistries block cytochrome P450-dependent monooxygenases. This inhibits oxidation of ent-kaurene to ent-kaurenoic acid, and therefore reduces formation of biologically active GAs (Rademacher, 2000).

FL and PB are not readily absorbed by leaf tissue or turfgrass crowns, but instead are translocated acropetally from root absorption. Irrigation or rainfall after treatment is required for these chemistries to reach roots for uptake (Anonymous, 2013; Anonymous, 2018b). Class B PGRs have half-lives of multiple months in soil, and are slow to initially regulate but have longer periods of regulation than class A PGRs (Diesburg and Christians, 1989; Kreuser, 2018; Kreuser and Soldat, 2011; Rademacher, 2000; Reasor et al., 2018). Class B inhibitors may have seedhead suppression capabilities, primarily on annual bluegrass (Bigelow et al., 2007; Fagerness and Penner, 1998a; Johnson and Murphy, 1995; Johnson and Murphy, 1996; Woosley et al., 2003).

Class C PGRs are cell division inhibitors, and include mefluidide, maleic hydrazide and amidochlor (Kreuser, 2015). Class C PGRs are more phytotoxic and their duration of growth suppression is shorter than class A and B PGRs, requiring more frequent applications (Diesburg and Christians, 1989; Haguewood et al., 2013).

Class D PGRs are herbicides used at less than lethal rates. Examples of class D compounds used for turfgrass growth suppression and seedhead control are glyphosate and imazapic (Kreuser, 2015; McCarty, 2018). Specific concentrations of must be used to effectively control growth without causing severe phytotoxicity or plant death.

Class E PGRs are phytohormones with no effect on gibberellin biosynthesis, and includes ethephon (EP) (Kreuser, 2015). EP is foliar absorbed, and which is quickly converted to ethylene. Ethylene is a natural signal of injury or stress and interferes with growth processes (McCarty, 2018; McCullough and Sidhu, 2014; Taiz and Zeigler, 2010).

Lastly, Class F PGRs are natural growth regulators. Compounds in this category include natural plant hormones and commercial biostimulant products utilizing their qualities, such as seaweed extract providing cytokinins (Kreuser, 2015; Zhang and Ervin, 2004).

Various PGRs' effects on 'Diamond' zoysiagrass' phytotoxicity or seedhead suppression has not been fully investigated (McCullough et al., 2017; Menchyk et al., 2014). TE has been a primary focus of PGR studies on 'Diamond' zoysiagrass (Atkinson et al., 2012; Qian and Engelke, 1999) but few studies have analyzed seedhead suppression (Patton et al., 2018). PB and EP have all proven to reduce annual bluegrass seedhead production and growth, mostly in creeping bentgrass (Askew, 2017; Haguewood et al., 2013; Johnson and Murphy, 1995; Johnson and Murphy 1996; Kane and Miller, 2003; Patton et al., 2018; Woosley et al., 2003) but none of these PGRs' effects on 'Diamond' zoysiagrass' seedhead production have been reported. Simazine (SI) has been observed to reduce seedhead production in 'Diamond' zoysiagrass when applied as a pre-emergent herbicide (Donald Garrett, personal communication, 2018). Imazapic and imazamox suppressed 'Meyer' zoysiagrass and 'Zenith' zoysiagrass seedhead formation >90% but phytotoxicity is common (Brosnan et al., 2012). McCullough et al. (2014) reported flucarbazone-sodium reduced seashore paspalum seedheads >80%. Older chemistries such as maleic hydrazide, mefluidide and amidochlor were not included in this study due to modern putting green standards, chemistries being taken off the market, and shorter durations of these chemistries in plants (Diesburg and Christians. 1989; Gaussoin et al., 1997).

Trinexapac-ethyl (TE)

Trinexapac-ethyl is a class A gibberellin biosynthesis inhibiting PGR, and is currently considered turfgrass industry's standard PGR (Kreuser, 2015). Primo Maxx (Syngenta, Greensboro, NC) is produced in liquid form containing 11.3% TE (Anonymous, 2015).

Fagerness and Penner (1998b) reported TE was 94% leaf sheath absorbed, 70% leaf blade absorbed, and 5% root absorbed by Kentucky bluegrass (*Poa pratensis* L.) within 24 h, with foliar absorption mostly taking place within 1 h. This study also found roots translocated only 50% of absorbed chemistry, while sheaths readily translocated TE acropetally to foliar tissue, and leaf blades retained or translocated TE to other foliar tissue (Fagerness and Penner, 1998b).

TE is commonly utilized to inhibit vertical shoot growth, shorten internode lengths and increase ball roll distances on putting surfaces (McCullough et al., 2006; McCullough et al., 2007; McCarty et al., 2011). However, Menchyk et al. (2014) found TE applied to 'Diamond' zoysiagrass did not significantly increase ball roll distance across various nitrogen regiments, possibly due to insufficient rates. TE reduced 'Tifway' bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy) vertical shoot growth while promoting lateral growth (Fagerness et al., 2002). TE positively affects turfgrass rooting (Beasley et al., 2005; McCarty et al., 2011), total nonstructural carbohydrate levels (Qian et al., 1998), and cell density in turfgrass leaves, possibly leading to increased traffic tolerance (Heckman et al. 2005).

TE reduced tissue production but a 'rebound effect' of growth may occur after TE had been fully metabolized in ‘Tifway’ bermudagrass (Fagerness and Yelverton, 2000) and in creeping bentgrass (Kreuser and Soldat, 2011). Trinexapac-acid, the plant-active form of TE, was significantly reduced in Kentucky bluegrass and creeping bentgrass by increased air temperatures (Beasley et al. 2005). Studies have developed GDD models for TE on creeping bentgrass putting greens (Kreuser and Soldat, 2011) and on ultradwarf bermudagrass putting greens (Reasor et al., 2018) to achieve efficient season long growth suppression and prevent a ‘rebound effect’.

TE applications to ‘Diamond’ zoysiagrass reduce elongation and prevent scalping in shade environments significantly improved turfgrass quality (Atkinson et al., 2012; Qian et al., 1998). This interaction is vital as ‘Diamond’ zoysiagrass is commonly utilized in high shade conditions where ultradwarf bermudagrass greens are not viable (Bunnel et al., 2005). Ervin et al. (2002) reported ‘Meyer’ density was also increased by using TE in shade conditions. TE is also commonly paired with other PGRs to mitigate undesirable phytotoxicity (Bigelow et al., 2007; Haguewood et al., 2013; Kane and Miller, 2003). TE is ineffective for controlling annual bluegrass seedhead production (Fagerness et al. 1998a), and Qian (1998) observed TE visually increased ‘Diamond’ zoysiagrass’ seedhead production.

Prohexadione calcium (PC)

Prohexadione calcium is a class A gibberellin biosynthesis inhibiting PGR, most similar to TE. Anuew (Nufarm Americas, Alsip, IL) is produced in an extruded granule formula with 27.5% PC (Anonymous, 2018a).

Prohexadione calcium is quickly absorbed through leaves and crown and is then readily translocated throughout the plant (Evans et al., 1999). PC was reported to suppress creeping bentgrass growth for nearly identical GDD as TE at multiple application rates (Kreuser, 2015; Kreuser and Soldat, 2011). Beam (2004) reported shoot growth reduction from PC was comparable to TE in bermudagrass, Kentucky bluegrass, perennial ryegrass (*Lolium perenne* L.) and zoysiagrass at varying rates. Beam (2004) also reported repeat PC treatments reduced annual bluegrass greater than 50% and was equivalent to PB at 3, 6, 8 and 17 wk after treatment. In ultradwarf bermudagrass putting greens, Reasor et al. (2018) reported PC's peak suppression was 95 GDD while TE's peak was 172 GDD, a more rapid plant response to PC. However, duration of growth suppression was 216-230 GDD for TE and 120-126 GDD for PC, revealing reduced longevity (Reasor et al., 2018). Minor differences are likely due to PC's active acid form being present once calcium is dissolved in water and is primarily acropetally translocated. Conversely, ester TE is translocated systemically to roots before being metabolized to its active acid form and then translocating acropetally (Beam and Askew, 2007).

Flurprimidol (FL)

Flurprimidol is a class B gibberellic acid biosynthesis inhibitor. Cutless (SePRO, Carmel, IN) contains 16% FL and is produced as a micro-emulsion concentrate liquid (Anonymous, 2018b). FL is primarily crown and root absorbed and is translocated acropetally to foliage, requiring irrigation or rainfall to be effective (Anonymous, 2018b).

Multiple studies found FL provides an increase in turfgrass color and density, while reducing vegetative growth in bermudagrass for up to 8 wk (Johnson, 1992; Johnson, 1994; Totten et al., 2006). Lowe and Whitwell (1999) reported FL reduced the height of bermudagrass and bahiagrass by 33% and 25%, respectively. Increasing FL rates on creeping bentgrass and annual bluegrass increased chlorophyll content (Gaussoin et al., 1997). FL also reduced annual bluegrass in creeping bentgrass fairways (Bigelow et al., 2007) and putting greens (Johnson and Murphy, 1995; Johnson and Murphy, 1996). FL applied to Kentucky bluegrass reduced growth by 16% on average and peak growth suppression was observed 10 wk after treatment, an increased longevity over class A PGRs (Diesburg and Christians, 1989; Kreuser and Soldat, 2011).

McCullough et al. (2005a) reported FL had 43% root mass reduction in 'Champion' bermudagrass putting greens and McCarty et al. (2011) reported a 25% reduction of root length density in 'Tifeagle' bermudagrass. Bunnell (2003) reported FL decreased perennial ryegrass and Kentucky bluegrass seed production

Paclobutrazol (PB)

Paclobutrazol is a class B gibberellin biosynthesis inhibitor. Trimmit (Syngenta, Greensboro, NC) is produced in a suspension concentrate formulation with 22.9% PB (Anonymous, 2013). PB is primarily crown and root absorbed, requiring irrigation or rainfall to be available to plants (Anonymous, 2013).

Paclobutrazol reduced bermudagrass growth up to 5 wk after a single application (Johnson, 1990). PB reduced bermudagrass height by 30%, but only reduced bahiagrass height by 5% (Lowe and Whitwell, 1999). Suppression from PB outlasted TE in

Kentucky bluegrass (Beasley and Branham, 2007; Diesburg and Christians, 1989). Peak suppression for Kentucky bluegrass treated with PB was 5 wk after treatment (Diesburg and Christians, 1989). A GDD (base of 0°C) model for PB on creeping bentgrass putting greens found increasing rates impacted growth suppression levels, but duration was only marginally extended (Kreuser et al., 2018). Kreuser et al. (2018) also reported the highest labeled rate of PB to be much more effective in reducing clipping yield than the highest labeled high rate of TE. Combinations of each chemistry at half rates did not hasten suppression or minimize 'rebound effect' but provided the greatest clipping yield reductions. PB did not reduce root biomass of creeping bentgrass (Fagerness and Yelverton, 2001) or 'Tifeagle' bermudagrass putting greens (McCullough et al., 2005a), but severely reduced root length in Kentucky bluegrass (Beasley and Branham, 2007).

Flowering and growth of annual bluegrass is reduced following applications of PB, and is a common method to slowly reduce annual bluegrass populations in creeping bentgrass putting greens (Johnson and Murphy, 1995; Johnson and Murphy, 1996; Woosley et al., 2003). King et al., (2006) reported PB prevented flowering in darnel ryegrass.

Ethephon (EP)

Ethephon is a class E phytohormone PGR. Proxy (Bayer, Research Triangle Park, NC) is produced as a liquid and contains 21.7% EP (Anonymous, 2018c). EP is foliar absorbed by plants and combines with water to produce ethylene gas. Ethylene gas inhibits growth by reducing hypocotyl elongation, and selectively aborts flowering

(McCullough and Sidhu, 2014; Serek and Reid, 2000).

McCullough (2017) reported ‘Diamond’ zoysiagrass possessed a higher tolerance to EP than both bermudagrass and seashore paspalum. EP applied to bermudagrass reduced plant height, increased rooting under drought stress, and increased tillering, but caused necrosis and chlorotic striping (Shatters et al., 1998). Ervin and Ok (2001) reported ‘Meyer’ zoysiagrass treated with EP and TE had similar growth suppression responses. However, studies have also documented EP is not as effective in reducing shoot growth as class A and B PGRs and may widen leaf blades (Diesburg and Christians, 1989; McCullough et al., 2006; McCullough et al., 2005c).

Ethephon applications reduced root length density of perennial ryegrass (Jiang and Fry, 1998), dry root mass in creeping bentgrass (McCullough et al., 2006), and root length and mass of ‘Tifeagle’ bermudagrass putting greens (McCullough et al. 2005c). These results raise concerns of EP use on putting greens, especially during or leading up to periods of plant stress.

Fall applications of EP to ‘Meyer’ zoysiagrass suppressed seedhead formation the following spring, suggesting residual effects (Patton et al., 2018). A similar interaction was reported by Askew (2017) in creeping bentgrass where winter applied EP increased annual bluegrass control in spring. EP reduces seedheads and growth of annual bluegrass, encouraging creeping bentgrass to spread into void areas (Gelernter and Stowell, 2001; Haguewood et al., 2013; Kane and Miller; 2003; McCullough and Sidhu, 2014). Turfgrass managers combine EP with class A PGRs to reduce phytotoxicity while retaining seedhead inhibition traits (Haguewood et al., 2013).

Simazine (SI)

Simazine is a photosystem II inhibiting herbicide, classified as a class D PGR. Princep 4L (Syngenta, Greensboro, NC) is formulated as a liquid and contains 41.9% SI (Anonymous, 2014). SI is primarily root absorbed by plants, requiring irrigation or rainfall to become available to plants (Cobb and Reade, 2011).

SI is commonly used for pre-emergence, early post-emergence and establishment weed control in C_4 turfgrass systems (McElroy and Martins, 2013). SI inhibits electron flow along photosystem II (PSII), specifically the ‘Hill reaction’ (Cobb and Reade, 2011). This results in active oxygen species causing membrane protein damage and slow plant death. Weeds must be exposed to sunlight for simazine’s active form to become effective (Cobb and Reade, 2011). It is common for metabolism resistance of SI to occur through higher concentrations of cytochrome P450 monooxygenase family of enzymes (Cobb and Reade, 2011) and a single gene mutation of a protein in PSII complexes can create target site resistance to PSII inhibitors (Kelly et al., 1999).

Fry et al. (1986) reported SI had varying effects on *Zoysia* spp. plug roots during establishment but SI increased stolon production. SI has proven effective for controlling many problematic turfgrass weeds (Fry et al., 1986; McElroy and Martins, 2013), but annual bluegrass has developed resistance (Hutto et al., 2004). SI applied to *Poaceae* species increased yields, protein content, and nitrate reductase activity (Ries and Wert, 1972; Ries et al., 1970; Tweedy and Ries, 1967).

CHAPTER TWO

'DIAMOND' ZOYSIAGRASS (*Zoysia matrella* (L.) Merr.) PUTTING GREEN SEEDHEAD CONTROL AND RESPONSE TO PLANT GROWTH REGULATORS

Introduction

'Diamond' zoysiagrass (*Zoysia matrella* (L.) Merr.) has become an increasingly popular choice for putting greens. Its C_4 physiology is able to withstand drought and heat stress more efficiently than C_3 species used for putting greens. 'Diamond' zoysiagrass when compared to other C_4 species used for putting greens has superb shade tolerance (Atkinson et al., 2012; Qian et al., 1998). However, 'Diamond' zoysiagrass typically produces seedheads in spring and fall on putting greens and create undesirable aesthetics, affect ball roll smoothness and possibly distance. Cultural practices to remove seedheads such as grooming, brushing and increased mowing frequency are not completely effective (Personal communication Donald Garrett, 2018). Plant growth regulators applied to annual bluegrass have reduced seedhead production while maintaining high turfgrass quality.

Materials and Methods

A field experiment was conducted from April 2018 to November 2019 on a 'Diamond' zoysiagrass practice green at the Walker Golf Course at Clemson University. A first objective of this study was to evaluate methods for predicting initial seedhead emergence. Second and third objectives were to determine if turf quality (TQ) and normalized difference of vegetative index (NDVI) decreased in response to various PGRs previously undocumented at putting green heights. Fourth and fifth objectives were to determine if PGRs decreased seedhead percent coverage and seedhead count. A sixth objective was to determine if PGR applications reduced root mass.

This ‘Diamond’ zoysiagrass green was established in June 2013 from sod over a converted creeping bentgrass green with USGA soil mix originally constructed in 1995 (Donald Garrett, personal communication). Sunrise-sunset times and temperature data were collected and used to calculate photoperiod and accumulated growing degree days (GDD) during the study (NOAA; Sunrise Sunset). Plots were not re-randomized from year 1 to 2, as research reported fall applications to influence spring seedhead production (Patton et al., 2018). Treatments were applied once in each spring and fall using a pressurized CO_2 backpack boom sprayer with a carrier volume 189.5 L/ha through 8003 flat fan nozzles (Tee jet, Spraying Systems Co., Roswell, GA). Overhead irrigation equivalent to 1.25 cm was applied 4 h after treatment to incorporate root absorbed PGRs. Rates for treatments were derived from *Zoysia* spp. with higher mowing heights, ultradwarf bermudagrass and observational research (L.B. McCarty, personal communication, 2018; Donald Garrett, personal communication, 2018). Treatments (Table 1) included trinexapac-ethyl (TE), paclobutrazol (PB), ethephon (EP), simazine (SI), flurprimidol (FL) and prohexadione-calcium (PC). Separate treatments of each PGR + TE were included to observe phytotoxicity effects. All treatments also included a non-ionic surfactant (NIS) (Harrell’s SprayMAX, Lakeland, FL) at a 0.25% volume volume^{-1} rate.

Table 1. Treatments and rates for 'Diamond' zoysiagrass putting green seedhead control and response to plant growth regulators study.

Treatment[a]	Standard Rate (lb a.i. $acre^{-1}$)	Metric Rate (g a.i. ha^{-1})
Untreated	–	–
TE (Primo Maxx 1L)	0.0366	41
PB (Trimmit 2SC)	0.0598	67
PB + TE	0.0598 + 0.0366	67 + 41
EP (Proxy 2L)	3.0816	3,454
EP + TE	3.0816 + 0.0366	3,454 + 41
SI (Princep 4L)	0.8922	1,000
SI + TE	0.8922 + 0.0366	1,000 + 41
PC (Anuew 27.5WP)	0.1035	116
PC + TE	0.1035 + 0.0366	116 + 41
FL (Cutless MEC 1L)	0.0834	93.5
FL + TE	0.0834 + 0.0366	93.5 + 41

[a] All treatments also included a NIS at a 0.25% volume $volume^{-1}$ rate.

Plots were mowed daily by Walker Golf Course staff from 2.54 to 3.175 mm. Solid tine aerification, vertical mower grooming, and topdressing were all performed uniformly throughout this study. Core aeration was performed using 1.27 cm tines with 2.54 x 2.54 cm spacing on 25 June 2018 and 21 June 2019. Fertilization was applied via foliar application equivalent to 9.8 g N m^{-2} $month^{-1}$ during rating dates. Fungicide applications were applied uniformly across plots, but no plant growth regulating products were applied during rating dates.

Statistical Design

Statistical design was randomized complete block with four replications and repeated observations at different rating dates. Data were then subjected to an overall ANOVA (Tables 2, 3 and 4) to determine treatment effects across all rating dates and any treatment by rating date interactions. Since treatment by rating date interactions were significant, rating data were subjected to ANOVA at each individual rating date to assess treatment effects. Means were separated using Fisher's protected LSD at a significance level of 0.05. Due to the variability of soil data, root mass means were deemed significant below 0.1, increasing chance for type I errors. Treatment and timing effects were also studied with contrasts to more confidently determine treatment effects by reducing total chances for type I error. Data were analyzed using JMP Pro 14.1 software (SAS Institute Inc.; Cary, NC, USA).

Table 2. Analysis of variance (ANOVA) table of a randomized complete block design for turf quality (TQ) and normalized difference of vegetative index (NDVI) in 'Diamond' zoysiagrass putting green seedhead control and response to plant growth regulators study.

Source of Variation	df[a]	TQ	NDVI
Treatment	11	***	NS
Block Error	3	NS	NS
Treatment x Block Error	33	NS	*
Season	3	***	***
Rating Date (Season)	16	***	***
Treatment x Season	33	***	***
Treatment x Rating Date (Season)	176	***	*
Error	685		
Corrected Total	959		

[a] Degrees of freedom
* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.05 probability level.

Table 3. Analysis of variance (ANOVA) table of a randomized complete block design for seedhead coverage and seedhead count in 'Diamond' zoysiagrass putting green seedhead control and response to plant growth regulators study.

Source of Variation	df[a]	Seedhead Coverage	Seedhead Count
Treatment	11	***	***
Block Error	3	NS	NS
Treatment x Block Error	33	NS	NS
Season	3	***	***
Rating Date (Season)	12	***	***
Treatment x Season	33	***	***
Treatment x Rating Date (Season)	132	***	***
Error	540		
Corrected Total	767		

[a] Degrees of freedom
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.05 probability level.

Table 4. Analysis of variance (ANOVA) table of a randomized complete block design for root mass count in 'Diamond' zoysiagrass putting green seedhead control and response to plant growth regulators study.

Source of Variation	df[a]	Root Mass
Treatment	11	NS
Block Error	3	NS
Treatment x Block Error	33	NS
Season	3	***
Treatment x Season	33	NS
Error	108	
Corrected Total	191	

[a] Degrees of freedom
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.1 probability level.

Measurements

Initial seedhead production was scouted in untreated control plots and recorded each season. These dates were related to the photoperiod for that day, defined as hours between initial sunrise and full sunset in Clemson, SC (SS, 2019). Dates were also compared to the accumulated growing degree days (GDD) (base 10°C) calculated using the equation,

$$\mathrm{DD} = \frac{(Max\ Temp + Minimum\ Temp)}{2} - 10$$

where max and minimum temperatures were collected from NOAA using Clemson University's station (NOAA, 2019). Turf quality (TQ) ratings were made on a 1 to 9 scale, with 9 being dense dark green grass and 1 being completely dead grass. Quality ratings below 7 were considered unacceptable for putting surfaces. Normalized difference of vegetative index (NDVI) was measured with a Field Scout TCM 500 NDVI Turf Color Meter (Spectrum Technologies, Bridgend, United Kingdom). Three random samples were taken within each plot and averaged. These values range from 0 – 0.999, and NDVI is calculated using the formula

$$\mathrm{NDVI} = \frac{\mathrm{R780} - \mathrm{R670}}{\mathrm{R780} + \mathrm{R670}}$$

where R780 and R670 are designated as the measured reflectance of near-infrared radiation (780 nm) and visible red radiation (670 nm) (Bremer et al. 2011; Trenholm et al. 1999). TQ and NDVI were rated 3, 7, 14, 28, and 42 days after treatment (DAT). Seedhead coverage was estimated as a percentage where 0 was no coverage and 100 was entire plot coverage. Seedheads were also counted within a 7 x 7 cm random sample within each plot. Seedhead percent coverage and counts were rated 14, 28, 42, and 56DAT. Three random 2.54 cm core samples of 10 cm depth were harvested from each plot and combined at 56DAT. Roots were severed uniformly below thatch and separated. Roots were then dried for 72 h at 60°C, and root mass was determined via loss by ignition in a muffle furnace at 525°C (Snyder and Cisar, 2000).

Results and Discussion

Seedhead Emergence

In Clemson, SC initial seedhead emergence was observed in spring 2018 at 158 GDD, and in spring 2019 at 110 GDD. In fall 2018, initial emergence was at 2433 GDD and in fall 2019, initial emergence was at 2372 GDD (Figure 1; Table 5). These results are reasonably similar to findings by McCullough (2017), who reported initial emergence in fairway height 'Diamond' zoysiagrass at 167 and 196 GDD in two spring seasons and 1949 and 2230 GDD in two fall seasons. Differences in height of cut likely attributed to variations between studies. GDD proved to be a consistent metric to predict seedhead emergence.

In Clemson, SC initial emergence occurred when photoperiod was 12.4 h in spring 2018 and 12.4 h in spring 2019. Initial emergence occurred when photoperiod was 12.3 h in fall 2018, and 12.4 h in fall 2019. These results also support McCullough et al. (2017) who reported initial emergence at 12.8 h in spring and fall. Again, differences in height of cut likely influenced seedhead emergence timing. Photoperiod proved to be a simpler and consistent metric to predict initial seedhead emergence in this study (Figure 1; Table 5).

Peak fall seedhead production was more consistent in density and duration, observed in seedhead count. Fall seedheads were most abundant when photoperiod was between 11 and 12 h. Fall seedheads were somewhat persistent through winter months in a dormant state and may become initial spring seedheads (data not shown). Spring seedheads were sporadic and lacked overall density. Managers can use these metrics to time applications.

Table 5. Initial seedhead emergence dates for ‘Diamond’ zoysiagrass putting green, accumulated growing degree days (GDD) and photoperiod of Clemson, SC during 2018 and 2019.

Initial Seedhead Emergence[a]	Accumulated GDD (Base 10°C)[b]	Photoperiod[c]
26 March 2018	158	12.4
17 September 2018	2433	12.3
27 March 2019	110	12.4
16 September 2019	2444	12.4

[a] First date a seedhead was observed in control plots.
[b] Sum of GDD from 1 January until date of emergence.
[c] Photoperiod on date of emergence.

Turf Quality

Turf quality (TQ) had a significant date by treatment interaction, p-value <0.0001, warranting focus on individual dates (Tables 6 and 7). In spring 2018 at 7DAT, EP + TE decreased TQ to 6, significantly below all treatments. At 14DAT, PC, PC + TE, FL + TE, and PB + TE, all decreased TQ to 6, and EP + TE decreased TQ to 5.75, all significantly below untreated. Decreases in quality were due to discoloration and reductions in density. Results were not fully replicated in spring 2019, where at 7DAT, only EP and EP + TE significantly decreased to 6. No treatment significantly increased TQ over untreated during spring seasons.

In fall 2018, SI and SI + TE caused significant reductions. At 3DAT, SI and SI + TE decreased to 6, while EP + TE decreased to 6.8 compared to 7 for the untreated. At 7DAT, SI, SI + TE, and EP + TE decreased to 5.5, 5.3, and 6.5, respectively, compared to 7.3 for the untreated. At 14DAT, SI and SI + TE decreased to 6 and 5.3, respectively, compared to 7 for the untreated. At 28DAT, SI + TE decreased to 6.3 compared to 7 for the untreated. In fall 2019 at 3DAT, SI and SI + TE decreased to 6 compared to 7.3 for the untreated. At 7DAT, SI and SI + TE were similar to control, a stark difference from 2018.

Contrast tests were performed to determine effects of combinations of TE and PGRs. TQ was significantly higher for PGRs without the addition of TE in spring 2018 at 3, 7, and 14DAT, in fall 2018 at 14DAT, and in fall 2019 at 7DAT (Table 8). TQ was never significantly higher across all PGRs with the addition of TE.

Irrigation was unavailable to be applied exactly 4 h after treatment in 2018 due to golfers utilizing the area, and application conditions for 2019 were milder. Irrigation was deferred to 12 h after treatment. These factors ultimately increased phytotoxicity and thinning of SI containing treatments in 2018. This illustrates the importance of thorough and prompt incorporation of soil absorbed treatments. These results establish reasonable rates for these PGRs to be used on *Z. matrella* putting greens. No treatment increased TQ over untreated during the present study, but spring and fall timings are likely be less responsive to PGRs than summer months. The addition of TE to alternative PGRs never significantly improved TQ, likely providing excessive regulation.

Further research should investigate reduced rates and multiple applications to prevent phytotoxicity of SI. Application intervals and increased rates during summer months should also be studied for impacts on ball roll distance, clipping yield and TQ.

Table 6. Turf quality response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 13 April 2018 (3 days after treatment) (DAT) through 5 June 2018 (56DAT) and 21 March 2019 (3DAT) through 13 May 2019 (56DAT) in Clemson, South Carolina.

	Spring 2018					Spring 2019				
Treatment	3DAT	7DAT	14DAT	28DAT	42DAT	3DAT	7DAT	14DAT	28DAT	42DAT
					1 – 9 (Best)					
Untreated	6.5abc	6.8a	6.75a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	6.8bc
TE	7.0a	7.0a	6.75a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.3a
PC	7.0a	7.0a	6.0bc	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0ab
PC + TE	6.5abc	6.5ab	6.0bc	7.0a	7.0a	7.0a	6.8a	7.0a	7.0a	7.0ab
FL	7.0a	7.0a	6.5ab	7.0a	7.0a	7.0a	7.0a	6.5ab	6.8a	7.0ab
FL +TE	7.0a	6.8a	6.0bc	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	7.0ab
PB	6.8ab	7.0a	7.0a	7.0a	7.0a	7.0a	7.0a	6.8ab	7.0a	7.0ab
PB + TE	6.3bc	6.5ab	6.0bc	7.0a	7.0a	7.0a	6.8a	6.8ab	7.0a	7.0ab
EP	6.8ab	6.5ab	6.75a	7.0a	7.0a	6.5b	6.0b	6.5ab	6.8a	6.5c
EP + TE	6.0c	6.0b	5.75c	7.0a	7.0a	6.5b	6.0b	6.3b	7.0a	6.8bc
SI	7.0a	6.8a	7.0a	7.0a	7.0a	6.8ab	7.0a	6.8ab	7.0a	7.0ab
SI + TE	7.0a	7.0a	6.75a	7.0a	7.0a	6.8ab	7.0a	6.8ab	7.0a	7.0ab
LSD	0.5	0.6	0.5	0	0	0.5	0.3	0.6	0.3	0.5
Significance	**	*	***	NS	NS	NS	***	NS	NS	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.05 probability level.
§ Turf quality rated from 1 – 9 where 9 = best turf and values < 7.0 are unacceptable.

Table 7. Turf quality response means of 'Diamond' zoysiagrass putting green to various plant growth regulators from 9 September 2018 (3DAT) through 1 November 2018 (56DAT) and 12 September 2019 (3DAT) through 4 November 2019 (56DAT) in Clemson, South Carolina.

	Fall 2018					Fall 2019				
Treatment	3DAT	7DAT	14DAT	28DAT	42DAT	3DAT	7DAT	14DAT	28DAT	42DAT
					1 – 9 (Best)					
Untreated	7.0a	7.3a	7.0ab	7.0a	7.0a	7.3ab	7.0b	7.0a	7.0a	7.0a
TE	7.0a	7.3a	7.3a	7.0a	7.0a	7.0ab	7.0b	7.0a	7.0a	7.0a
PC	7.0a	7.3a	7.0ab	7.0a	7.0a	7.5a	7.0b	7.0a	7.0a	7.0a
PC + TE	7.0a	7.3a	6.0cd	7.0a	7.0a	7.3ab	7.0b	7.0a	7.0a	7.0a
FL	7.0a	7.0a	7.0ab	6.8a	7.0a	7.0ab	7.5a	7.0a	7.0a	7.0a
FL +TE	7.0a	7.3a	6.0cd	6.8a	7.0a	7.3ab	7.0b	7.0a	7.0a	7.0a
PB	7.0a	7.0a	7.0ab	7.0a	7.0a	7.5ab	7.5a	7.0a	7.0a	7.0a
PB + TE	7.0a	7.0a	6.3bc	7.0a	7.0a	7.3ab	7.0b	7.0a	7.0a	7.0a
EP	7.0a	6.8ab	7.3a	7.0a	7.0a	7.0ab	7.0b	7.0a	7.0a	7.0a
EP + TE	6.8b	6.5b	7.0ab	6.8ab	7.0a	6.8b	7.0b	7.0a	7.0a	7.0a
SI	6.0c	5.5c	6.0cd	6.8ab	7.0a	6.0c	7.0b	7.0a	7.0a	7.0a
SI + TE	6.0c	5.3c	5.3d	6.3b	7.0a	6.0c	6.8b	6.8b	6.8b	7.0a
LSD	0.2	0.6	0.8	0.5	0	0.7	0.3	0.2	0.2	0
Significance	***	***	***	NS	NS	***	***	NS	NS	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.05 probability level.
§ Turf quality rated from 1 – 9, where 9 = best turf and values < 7.0 are unacceptable.

Table 8. Contrasts of turf quality response means of 'Diamond' zoysiagrass putting green to plant growth regulator (PGR) treatments combined with trinexapac-ethyl (–) vs. PGRs alone (+) from 10 April 2018 through 4 November 2019 from 3 to 56 days after treatment (DAT) in Clemson, South Carolina.

Date	Estimate	Significance
Spring 2018 3DAT	0.350	**
7DAT	0.30	*
14DAT	0.550	***
28DAT	0	NS
42DAT	0	NS
Fall 2018 3DAT	0.050	NS
7DAT	0.050	NS
14DAT	0.750	***
28DAT	0.150	NS
42DAT	0	NS
Spring 2019 3DAT	0	NS
7DAT	0.10	NS
14DAT	−0.050	NS
28DAT	−0.10	NS
42DAT	−0.050	NS
Fall 2019 3DAT	0.10	NS
7DAT	0.250	**
14DAT	0.050	NS
28DAT	0.050	NS
42DAT	0	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.05 probability level.

Normalized Difference of Vegetative Index

Normalized difference of vegetative index (NDVI) had a significant date by treatment interaction, p-value 0.0203, warranting focus on individual dates. No spring rating date had significant differences in treatments for NDVI in 2018 or 2019. However, in fall 2019 at 3DAT, SI and SI + TE significantly decreased by 4 and 5%, respectively. At 7DAT, PC + TE, EP + TE, SI, and SI + TE significantly decreased by 2, 3, 4, and 7%, respectively. At 14DAT, SI, PC + TE, and SI + TE significantly decreased by 3, 3 ad 8%, respectively. These reductions in NDVI of SI containing treatments reflect reductions in

2018 TQ ratings. In fall 2019 at 7DAT, PC + TE and SI + TE significantly decreased by 2 and 4%, respectively. At 14DAT, FL + TE, EP + TE, PC + TE, and SI + TE significantly decreased by 3, 4, 4, and 6%, respectively.

Contrast tests illustrated NDVI was greater for PGRs alone in spring 2018 at 7 and 14DAT, in fall 2018 at 14 and 28DAT, and in fall 2019 at 14DAT. Addition of TE to other PGRs more consistently reduced NDVI, likely from over regulating growth.

These results illustrate these PGRs were able to be applied during early spring and fall seasons without drastic reductions in color, spring ‘greenup’ or winter survival. Treatments containing SI reduced NDVI in fall 2018 and 2019, but this period was significantly shorter in 2019 with thorough incorporation with irrigation. No treatments were able to significantly improve color over untreated.

Table 9. Normalized difference of vegetative index response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 13 April 2018 (3DAT) through 5 June 2018 (56DAT) and 21 March 2019 (3DAT) through 13 May 2019 (56DAT) in Clemson, South Carolina.

	Spring 2018					Spring 2019				
Treatment	3DAT	7DAT	14DAT	28DAT	42DAT	3DAT	7DAT	14DAT	28DAT	42DAT
					0 – 0.999 (Best)					
Untreated	0.62a	0.64bc	0.66abc	0.66c	0.71b	0.59ab	0.56ab	0.56abcd	0.65ab	0.68a
TE	0.62a	0.64abc	0.65bc	0.68abc	0.71b	0.58abc	0.55abc	0.56ab	0.67a	0.70a
PC	0.62a	0.64abc	0.65abc	0.68abc	0.71a	0.61a	0.57a	0.56abcd	0.66ab	0.69a
PC + TE	0.62a	0.63c	0.65bc	0.68ab	0.72a	0.58abc	0.55abc	0.56abcd	0.65ab	0.69a
FL	0.62a	0.65ab	0.67a	0.68ab	0.71a	0.56c	0.54bc	0.55bcd	0.65ab	0.69a
FL +TE	0.63a	0.64bc	0.66abc	0.67bc	0.71ab	0.59ab	0.55abc	0.56abc	0.66ab	0.69a
PB	0.63a	0.66a	0.66ab	0.68abc	0.71ab	0.56bc	0.55abc	0.57a	0.67a	0.68a
PB + TE	0.62a	0.64bc	0.65c	0.68ab	0.70b	0.58abc	0.54bc	0.56abcd	0.65ab	0.68a
EP	0.62a	0.65abc	0.66abc	0.69a	0.71ab	0.56bc	0.54bc	0.55bcd	0.64b	0.67a
EP + TE	0.62a	0.63c	0.65bc	0.68abc	0.72a	0.59abc	0.53c	0.55d	0.65ab	0.70a
SI	0.63a	0.64abc	0.66abc	0.69ab	0.71ab	0.57abc	0.54c	0.55cd	0.65ab	0.68a
SI + TE	0.62a	0.64abc	0.65abc	0.68ab	0.71ab	0.57abc	0.55abc	0.55bcd	0.66ab	0.69a
LSD	0.019	0.018	0.015	0.019	0.010	0.032	0.023	0.017	0.023	0.025
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
‡ NS, nonsignificant at 0.05 probability level.
§ NDVI values ranged from 0 – 0.999, where 0.999 = best possible color.

Table 10. Normalized difference of vegetative index response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 9 September 2018 (3DAT) through 1 November 2018 (56DAT) and 12 September 2019 (3DAT) through 4 November 2019 (56DAT) in Clemson, South Carolina.

	Fall 2018					Fall 2019				
Treatment	3DAT	7DAT	14DAT	28DAT	42DAT	3DAT	7DAT	14DAT	28DAT	42DAT
					0 – 0.999 (Best)					
Untreated	0.72a	0.73a	0.67ab	0.70ab	0.68ab	0.59abc	0.64ab	0.66a	0.66bcde	0.63ab
TE	0.72a	0.73ab	0.67ab	0.70ab	0.69a	0.59ab	0.63abc	0.65abc	0.68ab	0.65a
PC	0.72a	0.72ab	0.67ab	0.70ab	0.68a	0.59abc	0.64ab	0.65abc	0.68a	0.65ab
PC + TE	0.71a	0.71bc	0.65b	0.69b	0.67b	0.57bc	0.63bc	0.64bcd	0.67abc	0.64ab
FL	0.71a	0.72ab	0.67ab	0.70ab	0.69a	0.60a	0.65a	0.64abcd	0.66abcde	0.64ab
FL +TE	0.72a	0.73ab	0.67ab	0.70b	0.69a	0.59abc	0.65a	0.64bcd	0.67abcd	0.64ab
PB	0.71a	0.72ab	0.66ab	0.70ab	0.68ab	0.58abc	0.64ab	0.66ab	0.67abcd	0.64ab
PB + TE	0.71a	0.72ab	0.66ab	0.70b	0.68ab	0.59ab	0.64ab	0.64abcd	0.65cde	0.63b
EP	0.71a	0.72ab	0.68a	0.72a	0.68ab	0.59abc	0.64ab	0.65abc	0.65de	0.64ab
EP + TE	0.71a	0.71bc	0.67ab	0.71ab	0.68ab	0.58abc	0.64ab	0.63cd	0.67abcde	0.63ab
SI	0.69b	0.70c	0.65bc	0.70ab	0.69a	0.58abc	0.63bc	0.64abcd	0.66abcde	0.64ab
SI + TE	0.69b	0.68d	0.61c	0.68b	0.68ab	0.57c	0.62c	0.62d	0.65e	0.63ab
LSD	0.013	0.016	0.032	0.023	0.017	0.022	0.018	0.022	0.022	0.017
Significance	***	***	*	NS	NS	NS	*	*	NS	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.05 probability level.
§ NDVI values ranged from 0 – 0.999, where 0.999 = best possible color.

Table 11. Contrasts of normalized difference of vegetative index means of ‘Diamond’ zoysiagrass putting green to plant growth regulator (PGR) treatments combined with trinexapac-ethyl (–) vs. PGRs alone (+) from 10 April 2018 through 4 November 2019 from 3 to 56 days after treatment (DAT) in Clemson, South Carolina.

Date	Estimate	Significance
Spring 2018 3DAT	0.0005	NS
7DAT	0.0103	**
14DAT	0.0096	**
28DAT	0.0036	NS
42DAT	0.0004	NS
Fall 2018 3DAT	0.0022	NS
7DAT	0.0061	NS
14DAT	0.0162	*
28DAT	0.0118	*
42DAT	0.0060	NS
Spring 2019 3DAT	−0.0090	NS
7DAT	0.0047	NS
14DAT	−0.0010	NS
28DAT	0.0009	NS
42DAT	−0.0060	NS
Fall 2019 3DAT	0.0059	NS
7DAT	0.0051	NS
14DAT	0.0136	**
28DAT	0.0027	NS
42DAT	0.0062	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
‡ NS, nonsignificant at 0.05 probability level.

Seedhead Coverage

Seedhead coverage had a significant date by treatment interaction, p-value <0.0001, warranting focus on individual dates.

In spring 2018, seedhead emergence occurred before treatments were applied, negatively impacting seedhead control. However, at 14DAT in spring 2018, SI + TE reduced coverage by 10% from untreated. At 28DAT, SI + TE, PC + TE, FL, FL + TE, and EP reduced coverage by 8% from untreated, while SI and EP + TE reduced coverage by 10% from untreated. In spring 2019 at 14DAT, FL, PB, PB + TE, and EP reduced coverage by 8% from untreated.

In fall 2018, seedhead coverage was significantly different for all rating dates. In fall 2018 at 14DAT, SI + TE reduced coverage by 23% from untreated, while EP increased coverage by 15% over untreated. At 28DAT, SI, SI + TE, and EP reduced coverage by 38, 38 and 18%, respectively, from untreated. At 42DAT, SI and SI + TE reduced coverage by 38 and 35%, respectively, from untreated. At 56DAT, SI and SI + TE reduced coverage by 35 and 23%, respectively, from untreated.

In fall 2019 at 28DAT, SI and SI + TE both reduced coverage by 8% from untreated, while PC + TE and FL + TE increased coverage by ~8%, over untreated. At 42DAT, SI, SI + TE, EP, and FL reduced coverage by 46, 41, 18, and 15%, respectively, from untreated. At 56DAT, SI and SI + TE reduced coverage by 40 and 35%, respectively, from untreated, while PC + TE and FL + TE increased coverage by 18 and 15%, respectively, over untreated.

Contrast tests illustrated seedhead coverage in fall 2018 at 14DAT increased by 14% with the addition of TE to other PGRs. This effect needs further evaluation to be supported.

Table 12. Percentage seedhead coverage response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 24 April 2018 (14DAT) through 5 June 2018 (56DAT) and 1 April 2019 (14DAT) through 13 May 2019 (56DAT) in Clemson, South Carolina.

	Spring 2018				Spring 2019			
Treatment	14DAT	28DAT	42DAT	56DAT	14DAT	28DAT	42DAT	56DAT
				0 – 100% coverage plot^{-1}				
Untreated	25.0abc	10.0a	0a	0a	0a	12.5a	0a	0a
TE	30.0ab	10.0a	0a	0a	0a	12.5a	0a	0a
PC	22.5bcd	2.5bc	0a	0a	0a	12.5a	0a	0a
PC + TE	25.0abc	5.0abc	0a	0a	0a	7.5ab	0a	0a
FL	20.0cd	2.5bc	0a	0a	0a	5.0b	0a	0a
FL +TE	32.5a	2.5bc	0a	0a	0a	10.0ab	0a	0a
PB	22.5bcd	5.0abc	0a	0a	0a	5.0b	0a	0a
PB + TE	22.5bcd	7.5ab	0a	0a	0a	5.0b	0a	0a
EP	20.0cd	2.5bc	0a	0a	0a	5.0b	0a	0a
EP + TE	20.0cd	0c	0a	0a	0a	7.5ab	0a	0a
SI	17.5cd	0c	0a	0a	0a	7.5ab	0a	0a
SI + TE	15.0d	2.5bc	0a	0a	0a	10.0ab	0a	0a
LSD	9.4	6.6	0	0	0	6.8	0	0
Significance	*	*	NS	NS	NS	NS	NS	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
* Significant at the 0.05 probability level.
‡ NS, nonsignificant at 0.05 probability level.
§ Seedhead coverage percentage was determined visually by the portion of the plot with visible seedheads, where lower percentages are desirable.

Table 13. Percentage seedhead coverage response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 20 September 2018 (14DAT) through 1 November 2018 (56DAT) and 23 September 2019 (14DAT) through 4 November 2019 (56DAT) in Clemson, South Carolina.

	Fall 2018				Fall 2019			
Treatment	14DAT	28DAT	42DAT	56DAT	14DAT	28DAT	42DAT	56DAT
				0 – 100% coverage plot^{-1}				
Untreated	32.5bc	37.5bc	55.0a	65.0ab	0a	7.5bc	47.5a	45.0c
TE	22.5cd	37.5bc	40.0a	70.0a	0a	5.0cd	37.5abc	55.0abc
PC	40.0abc	52.5ab	42.5a	65.0ab	0a	13.8ab	45.0a	56.3abc
PC + TE	32.5bc	60.0a	52.5a	67.5ab	0a	16.3a	37.5abc	62.5a
FL	52.5ab	35.0bc	42.5a	60.0b	0a	13.8ab	32.5bc	52.5abc
FL +TE	35.0bc	30.0cd	45.0a	67.5ab	0a	15.0a	42.5ab	60.0ab
PB	40.0abc	30.0cd	42.5a	62.5ab	0a	13.8ab	47.5a	48.8bc
PB + TE	27.5cd	32.5cd	55.0a	65.0ab	0a	7.5bc	40.0abc	51.3abc
EP	57.5a	20.0d	45.0a	65.0ab	0a	5.0cd	30.0c	46.3c
EP + TE	37.5abc	35.0cd	47.5a	65.0ab	0a	7.5bc	40.0abc	48.8bc
SI	20.0cd	0e	12.5b	30.0c	0a	0d	1.25d	5.0d
SI + TE	10.0d	0e	15.0b	37.5c	0a	0d	6.25d	10.0d
LSD	20.1	17.3	15.3	9.6	0	6.7	11.3	11.6
Significance	**	***	***	***	NS	***	**	***

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at 0.05 probability level.
§ Seedhead coverage percentage was determined visually by the portion of the plot with visible seedheads, where lower percentages are desirable.

Table 14. Contrasts of percentage seedhead coverage means of ‘Diamond’ zoysiagrass putting green to plant growth regulator (PGR) treatments combined with trinexapac-ethyl (–) vs. PGRs alone (+) from 24 April 2018 through 4 November 2019 from 14 to 56 days after treatment (DAT) in Clemson, South Carolina.

Date	Estimate	Significance
Spring 2018 14DAT	−2.5	NS
28DAT	−1.0	NS
42DAT	0	NS
56DAT	0	NS
Fall 2018 14DAT	−4.0	NS
28DAT	13.5	**
42DAT	−6.0	NS
56DAT	−4.0	NS
Spring 2019 14DAT	0	NS
28DAT	−2.5	NS
42DAT	0	NS
56DAT	0	NS
Fall 2019 14DAT	0	NS
28DAT	−2.0	NS
42DAT	−2.0	NS
56DAT	−4.8	NS

** Significant at the 0.01 probability level.
‡ NS, nonsignificant at 0.05 probability level.

Seedhead Count

Seedhead count results had a significant date by treatment interaction, p-value <0.0001, warranting focus on individual dates.

In spring 2018, seedhead production began before treatments were applied on 10 April, likely reducing treatment effectiveness. However, in spring 2018 at 14DAT, SI + TE and EP + TE reduced count by 47 and 40%, respectively, from untreated.

Spring 2019 treatments were applied earlier, 18 March but this season was cooler than 2018 and may explain reduced seedhead production. Accumulated GDD at 10 April 2018 was 211, while 10 April 2019 was 175 (data not shown). These increased temperatures early in the season may have initiated earlier seedhead production. However, GDD at 14 May were 465 in 2018 and 500 in 2019. Recent findings report spring seedhead reductions from fall applications of EP (Patton et al., 2018), but poor seedhead production in spring 2019 lead to the current study being unable to support these findings.

In fall 2018 at 28DAT, SI and SI + TE decreased count by 97 and 98% from untreated, respectively, while PC increased count by 62% from untreated. At 42DAT, SI and SI + TE decreased count by 75 and 70% from untreated, respectively. At 56DAT, SI and SI + TE decreased count by 41 and 35% from untreated, respectively.

In fall 2019 at 28DAT, SI and SI + TE decreased count by 100 and 98% from untreated, respectively. At 42DAT, SI and SI + TE both decreased count by 98% from untreated. At 56DAT, SI and SI + TE decreased count by 96 and 92% from untreated, respectively.

In fall 2018 at 14DAT, contrast tests illustrated PGRs combined with TE reduced seedhead counts by 3 per sample over PGRs alone. The inconsistency of these findings indicates TE alone or in combination is not a sufficient seedhead reduction tool.

Seedhead control was most effectively achieved with treatments containing SI. All other treatments were unable to reduce seedhead count below untreated. Turfgrass managers should utilize SI to provide significantly less seedhead production for a large portion of the production period. Reapplication may be useful to extending control past 56DAT.

Further research should also be conducted using other chemistries than those in this study for seedhead control. Studies should investigate different application dates and re-application intervals to maximize seedhead control.

Table 15. Seedhead count response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 24 April 2018 (14DAT) through 5 June 2018 (56DAT) and 1 April 2019 (14DAT) through 13 May 2019 (56DAT) in Clemson, South Carolina.

	Spring 2018				Spring 2019			
Treatment	14DAT	28DAT	42DAT	56DAT	14DAT	28DAT	42DAT	56DAT
				count 7x7 cm-sample^{-1}				
Untreated	25.0ab	1.3ab	0a	0a	0a	1.0a	1.0a	1.3a
TE	28.0a	1.3ab	0a	0a	0a	1.8a	0.8ab	1.8a
PC	19.8abc	1.3ab	0a	0a	0a	1.8a	0.3ab	0.3a
PC + TE	21.3abc	1.3ab	0a	0a	0a	0.8a	0.8ab	0.3a
FL	17.5bc	0.5ab	0a	0a	0a	2.0a	0.8ab	1.0a
FL +TE	29.0a	1.3ab	0a	0a	0a	1.8a	0b	1.3a
PB	21.5abc	1.8a	0a	0a	0a	0.8a	0.3ab	1.0a
PB + TE	18.3bc	1.5ab	0a	0a	0a	1.0a	0.5ab	1.0a
EP	21.3abc	0.5ab	0a	0a	0a	1.8a	0.5ab	0.3a
EP + TE	15.0c	0.8ab	0a	0a	0a	0.3a	0.8ab	0.8a
SI	15.5bc	0.3b	0a	0a	0a	1.0a	0b	1.8a
SI + TE	13.3c	0.8ab	0a	0a	0a	2.0a	1.0a	1.3a
LSD	9.7	1.3	0	0	0	2.0	1.0	1.5
Significance	*	NS	NS	NS	NS	NS	NS	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
* Significant at the 0.05 probability level.
‡ NS, nonsignificant at 0.05 probability level.
§ Seedheads were counter in each sample, where lower counts are desirable.

Table 16. Seedhead count response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 20 September 2018 (14DAT) through 1 November 2018 (56DAT) and 23 September 2019 (14DAT) through 4 November 2019 (56DAT) in Clemson, South Carolina.

	Fall 2018				Fall 2019			
Treatment	14DAT	28DAT	42DAT	56DAT	14DAT	28DAT	42DAT	56DAT
				count 7x7 cm-sample^{-1}				
Untreated	4.0bc	68.5bcd	114.3abc	130.5abc	0a	27.5abcd	81.0ab	157.3ab
TE	3.8bc	79.5abc	131.8a	151.0a	0a	22.8cd	87.5a	181.5a
PC	9.8a	111.3a	127.3a	121.8bc	0a	36.3abc	92.75a	180.0a
PC + TE	3.5bc	102.3ab	103.3abc	139.5abc	0a	21.5cd	89.5a	169.0ab
FL	8.5abc	57.5cd	96.3bc	123.8abc	0a	26.8bcd	82.0ab	152.3ab
FL +TE	5.3abc	68.5bcd	112.8abc	147ab	0a	47.0ab	89.0a	183.0a
PB	6.5abc	37.5de	83.8c	117.3c	0a	47.8a	76.8ab	160.5ab
PB + TE	2.5c	58.8cd	120.3ab	130.0ab	0a	23.0cd	73.8ab	163.3ab
EP	6.5abc	30.3de	123.5ab	128.5abc	0a	8.0de	60.0b	137.0b
EP + TE	5.8abc	30.3de	126.3ab	121.5bc	0a	11.0de	61.5b	137.5b
SI	2.3c	2.3e	28.8d	76.5d	0a	0e	1.3c	6.8c
SI + TE	1.5c	1.5e	34.8d	84.5d	0a	0.5e	1.8c	12.8c
LSD	5.7	41.0	30.6	28.9	0	20.7	24.1	41.1
Significance	NS	***	***	***	NS	***	***	***

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

*** Significant at the 0.001 probability level**.**

‡ NS, nonsignificant at 0.05 probability level.

§ Seedheads were counter in each sample, where lower counts are desirable.

Table 17. Contrasts of seedhead count means of 'Diamond' zoysiagrass putting green to plant growth regulator (PGR) treatments combined with trinexapac-ethyl (–) vs. PGRs alone (+) from 24 April 2018 through 4 November 2019 from 14 to 56 days after treatment (DAT) in Clemson, South Carolina.

Date	Estimate	Significance
Spring 2018 14DAT	−0.3	NS
28DAT	−0.3	NS
42DAT	−0.1	NS
56DAT	0	NS
Fall 2018 14DAT	3.0	*
28DAT	−4.5	NS
42DAT	−7.6	NS
56DAT	−11.0	NS
Spring 2019 14DAT	0	NS
28DAT	0.3	NS
42DAT	−0.3	NS
56DAT	−0.1	NS
Fall 2019 14DAT	0	NS
28DAT	3.2	NS
42DAT	−0.6	NS
56DAT	−5.8	NS

* Significant at the 0.05 probability level.
‡ NS, nonsignificant at 0.05 probability level.

Root Mass

Root mass was not significantly different for any treatment during any season (Table 18). Root mass was significantly affected by season, p-value <0.0001, with greater mass in spring 2018 but were not repeated in spring 2019. This could be due to considerable differences in sample collection timing, 5 June 2018 vs. 13 May 2019, with vastly different accumulated GDD, 772 vs. 494. McCullough (2005c) reported significant decreases in 'Tifeagle' bermudagrass putting greens from EP applications, but those results were not repeated in the present study. An extended period between treatment application and sampling, 56 days, could have allowed roots to recover.

Table 18. Root mass response means of ‘Diamond’ zoysiagrass putting green to various plant growth regulators from 56 days after treatment 5 June 2018, 1 November 2018, 13 May 2019 and 4 November 2019 in Clemson, South Carolina.

	2018		2019	
Treatment	Spring	Fall	Spring	Fall
	g root mass plot-sample^{-1}			
Untreated	0.56b	0.49a	0.52a	0.49a
TE	0.70ab	0.47a	0.59a	0.49a
PC	0.77a	0.51a	0.56a	0.53a
PC + TE	0.75ab	0.52a	0.56a	0.53a
FL	0.86a	0.51a	0.47a	0.51a
FL +TE	0.75ab	0.51a	0.56a	0.53a
PB	0.77a	0.49a	0.56a	0.54a
PB + TE	0.83a	0.48a	0.58a	0.49a
EP	0.70ab	0.53a	0.53a	0.43a
EP + TE	0.84a	0.49a	0.56a	0.47a
SI	0.74ab	0.48a	0.59a	0.52a
SI + TE	0.67ab	0.50a	0.49a	0.52a
LSD	0.20	0.12	0.14	0.15
Significance	NS	NS	NS	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.1).
‡ NS, nonsignificant at 0.1 probability level.
§ Root mass was determined via loss by ignition of samples which included 3 random 1.27 cm cores combined per plot, where higher weights are desirable.

Table 19. Contrasts of root mass means of ‘Diamond’ zoysiagrass putting green to plant growth regulators (PGRs) combined with trinexapac-ethyl (–) vs. PGRs alone (+) from 5 June 2018, 1 November 2018, 13 May 2019 and 4 November 2019 from 56 days after treatment in Clemson, South Carolina.

Date	Estimate	Significance
Spring 2018	−0.0010	NS
Fall 2018	0.0255	NS
Spring 2019	−0.008	NS
Fall 2019	−0.004	NS

‡ NS, nonsignificant at 0.1 probability level.

Contrasts

Contrast tests of means were performed to determine the effects of PGRs alone (-) and PGRs in combination with TE (Table 20). Spring TQ was lower than fall, and this was confirmed with spring NDVI being lower than fall. Both spring and fall seasons had higher TQ when PGRs were not combined with TE, and this is supported by fall NDVI being higher for PGRs alone.

Seedhead coverage and count were drastically higher for fall than spring, and neither metric was significantly impacted by presence or lack of TE with other PGRs. Seedhead coverage and count were greater in 2018 than 2019, likely due to fall 2019 14DAT having negligible seedhead presence.

These results emphasize seedhead control is more pertinent in fall than spring. This also offers flexibility with rates and other products as TQ and NDVI were greater in fall than spring. Treatment with TE or combinations with TE were unable to reduce seedhead production.

Table 20. Contrast tests of various means to determine how season and plant growth regulators (PGRs) used in combination with trinexapac-ethyl (–) compared to PGRs (+) alone affected measurements.

Contrasting Factors	Metric	Estimate	Significance
Spring (–) vs Fall seasons (+)	Turf Quality	0.0694	**
PGRs combined with TE (–) vs PGRs without TE (+)	Turf Quality	0.1063	***
Spring 2018 (–) vs Spring 2019 (+)	Turf Quality	0.0729	**
Spring PGRs combined with TE (–) vs PGRs without TE (+)	Turf Quality	0.0917	**
Fall 2018 (–) vs Fall 2019 (+)	Turf Quality	0.1701	***
Fall PGRs combined with TE (–) vs PGRs without TE (+)	Turf Quality	0.1208	***
Spring (–) vs Fall (+) seasons	NDVI	0.0156	***
PGRs combined with TE (–) vs PGRs without TE (+)	NDVI	0.0035	NS
Spring 2018 (–) vs Spring 2019 (+)	NDVI	−0.0490	***
Spring PGRs combined with TE (–) vs PGRs without TE (+)	NDVI	0.0003	NS
Fall 2018 (–) vs Fall 2019 (+)	NDVI	−0.056	***
Fall PGRs combined with TE (–) vs PGRs without TE (+)	NDVI	0.0072	**
Spring (–) vs Fall (+)	Seedhead Coverage	27.331	***
PGRs combined with TE (–) vs PGRs without TE (+)	Seedhead Coverage	−0.734	NS
Spring 2018 (–) vs Spring 2019 (+)	Seedhead Coverage	−4.635	***
Spring PGRs combined with TE (–) vs PGRs without TE (+)	Seedhead Coverage	−0.944	NS
Fall 2018 (–) vs Fall 2019 (+)	Seedhead Coverage	−19.56	***
Fall PGRs combined with TE (–) vs PGRs without TE (+)	Seedhead Coverage	−0.906	NS
Spring (–) vs Fall (+)	Seedhead Count	60.419	***
PGRs combined with TE (–) vs PGRs without TE (+)	Seedhead Count	−1.481	NS
Spring 2018 (–) vs Spring 2019 (+)	Seedhead Count	−4.6610	***
Spring PGRs combined with TE (–) vs PGRs without TE (+)	Seedhead Count	−0.0690	NS
Fall 2018 (–) vs Fall 2019 (+)	Seedhead Count	−14.02	***
Fall PGRs combined with TE (–) vs PGRs without TE (+)	Seedhead Count	−2.9	NS

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

‡ NS, nonsignificant at 0.05 probability level.

CHAPTER THREE

'DIAMOND' ZOYSIAGRASS (*Zoysia matrella* (L.) Merr.) SEEDHEAD CONTROL AND RESPONSE TO REPEAT APPLICATIONS OF PLANT GROWTH REGULATORS

Introduction

'Diamond' zoysiagrass (*Zoysia matrella* (L.) Merr.) putting green response to the plant growth regulator TE has been previously documented (Atkinson et al. 2012; Qian et al., 1998; Menchyk et al., 2014). However, alternative plant growth regulators (PGRs) such as PC, FL, PB and EP do not have labeled use rates for zoysiagrass putting greens. Repeat applications of plant growth regulators are often used to reduce clipping yield and increase ball roll distances. Concerns when using PGRs are turfgrass quality, clipping reduction, and possible rooting consequences. Repeat applications may also impact 'Diamond' zoysiagrass seedhead production.

Materials and Methods

An experiment was conducted from January 2019 to June 2019 at Clemson University Greenhouse Complex in Clemson, SC. This study's objectives were to determine if PGRs repeatedly applied reduced seedhead production, turf quality (TQ), normalized difference of vegetative index (NDVI), clipping weight and root mass.

'Diamond' zoysiagrass plugs 10.8 cm diameter and 15 cm depth were harvested in October 2018 from the nursery green at Walker Golf Course at Clemson University, which was originally constructed in June 2014 from sod over a converted creeping bentgrass green with USGA soil mix originally constructed in 1995 (personal communication with Don Garrett, 2018). Plugs were established in 15 cm diameter and 15 cm depth pots with USGA greens mix of 85 sand:15 peatmoss by volume (USGA, 2018). Plants were maintained with approximately 14 h photoperiod and a light intensity of 500 μmol m^{-2} s^{-1}, with day/night temperatures maintained near 28/19°C.

Due to no labeled use rates for zoysiagrass putting greens, use rates were derived from alternative zoysiagrass species with higher mowing heights, ultradwarf bermudagrass greens, and previous observational research (L.B. McCarty, personal communication, 2018; Donald Garrett, personal communication, 2018). Treatments were applied using an enclosed spray chamber (DeVries Manufacturing, Hollandale, MN) on a 4 wk interval beginning 2 January 2019 and included trinexapac-ethyl (TE), paclobutrazol (PB), ethephon (EP), simazine (SI), flurprimidol (FL) and prohexadione calcium (PC) (Table 21). All treatments also included a non-ionic surfactant (NIS) (Harrell's SprayMAX, Lakeland, FL) at 0.25% volume $volume^{-1}$. Foliar fertilization using Grigg Gary's Green 18-3-4 (Brandt Consolidated Inc. Springfield, IL) at 4.9 g N m^{-2} was also included in each application including untreated control plots. Pots were left to dry for 4 h after treatment application to satisfy leaf and sheath absorbed PGRs, then irrigated with 1.27 cm water to satisfy requirements for root absorbed PGRs. Pots were not mown after 2 January 2019 for seedhead counting and clipping measurement purposes. Pots were watered as needed to prevent wilting.

Table 21. Treatments and rates used in ‘Diamond’ zoysiagrass seedhead control and response to repeat applications of plant growth regulators study.

Treatment [a]	Standard Rate (lb a.i. $acre^{-1}$)	Metric Rate (g a.i. ha^{-1})
Untreated	–	–
TE (Primo Maxx 1MEC)	0.0366	41
PB (Trimmit 2SC)	0.0598	67
EP (Proxy 2L)	3.0816	3,454
SI (Princep 4L)	0.8922	1,000
PC (Anuew 27.5WP)	0.1035	116
FL (Cutless MEC 1.3L)	0.0834	93.5

[a] All treatments also included a NIS at a 0.25% volume $volume^{-1}$ rate, and 4.9 g N m^{-2}.

Statistical Design

Statistical design was randomized complete block with four blocks and two repetitions. Data were subjected to an overall ANOVA (Tables 22 and 23) to determine treatment effects across all rating dates and any treatment by rating date interactions. Since treatment by rating date interactions were often significant, the rating data were subjected to ANOVA at each rating date to assess treatment effects. Means were further studied using Fisher’s protected LSD at a significance level of 0.05. Due to the variability of soil data, root mass measurements were deemed significant below 0.1. Data were analyzed using JMP Pro 14.1 software (SAS Institute Inc.; Cary, NC, USA).

Table 22. Analysis of variance (ANOVA) table of a randomized complete block design for turfgrass quality (TQ), normalized difference of vegetative index (NDVI) and seedhead count p-values from 'Diamond' zoysiagrass seedhead control and response to repeat applications of plant growth regulators study.

Source of Variation	df[a]	TQ	NDVI	Seedhead Count
Treatment	6	*	**	***
Block (Repetition) Error	3	NS	NS	NS
Treatment x Block (Repetition) Error	18	*	*	NS
Repetition	1	NS	NS	NS
Treatment x Repetition	6	NS	NS	NS
Date	4	*	***	***
Treatment x Date	24	***	**	***
Error	217			
Corrected Total	279			

[a] Degrees of freedom
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at the 0.05 probability level.

Table 23. Analysis of variance (ANOVA) table of a randomized complete block design for clipping weight and root mass p-values from 'Diamond' zoysiagrass seedhead control and response to repeat applications of plant growth regulators study.

Source of Variation	df[a]	Clipping Mass	Root Mass
Treatment	6	***	NS
Block (Repetition) Error	3	NS	NS
Treatment x Block (Repetition) Error	18	NS	***
Repetition	1	NS	NS
Treatment x Repetition	6	NS	*
Error	21		
Corrected Total	55		

[a] Degrees of freedom
* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at the 0.05 probability level for Clipping Mass, and at the 0.1 probability level for Root Mass.

Measurements

All ratings were made at 4 wk intervals beginning 30 January 2019, or 4 weeks after initial treatment (WAIT). TQ was rated on a 1 to 9 scale with 9 being dense dark green grass and 1 being completely dead grass. TQ ratings below 7 were considered to be unacceptable. NDVI were rated with a Field Scout TCM 500 NDVI Turf Color Meter (Spectrum Technologies, Bridgend, United Kingdom). These values range from 0 – .999, and NDVI is calculated using the formula

$$\mathrm{NDVI} = \frac{\mathrm{R780} - \mathrm{R670}}{\mathrm{R780} + \mathrm{R670}}$$

where R780 and R670 are designated as the measured reflectance of near-infrared radiation (780 nm) and visible red radiation (670 nm) (Bremer et al. 2011; Trenholm et al. 1999). Seedheads were counted per pot. These ratings concluded 20WAIT. Clipping and root mass were collected 24WAIT. Clippings were removed with shears 3 mm above soil surface. Clippings were oven dried at 60°C for 72 h and weighed. Roots were harvested below the thatch layer, washed of soil, dried at 60°C for 72 h and weighed.

Results and Discussion

Turf Quality

For TQ a significant treatment by date effect occurred, p-value <0.0001, warranting focus on individual dates. No treatment by repetition effect was observed so results were combined.

All plots’ had TQ ratings of 7 at 0WAIT. TE steadily improved TQ with improved density and color with TQ of 8 at 20WAIT (Table 24). This supports Atkinson (2012) and Qian et al. (1998) who reported significant increases in TQ with TE applications. PC improved color and density similar to TE, with TQ of 7.9 at 20WAIT. FL also increased color and density, with a TQ of 7.8, but at 20WAIT had a decrease in TQ to 7.4 due to slight phytotoxicity (Table 24). SI at 16WAIT was similar to TE, PC and FL with TQ of 7.9, possessing a dark green color. However, by 20WAIT SI was slightly decreased, 7.9 to 7.5, due to reduced density when compared to all other treatments (Table 24). This illustrated SI had negligible growth regulation properties and is supported in clipping mass (Table 27).

Due to severe phytotoxicity, PB at 8, 12, 16, and 20WAIT had TQ of 7.4, 6.8, 6.8 and 7.0 at these weeks, significantly less than TE, PC, FL, and SI (Table 24). Reductions in TQ by FL at 20WAIT and PB at 8WAIT on could be due to longer suppression periods reported by Kreuser et al. (2018), and repeat applications resulted in excessive regulation. EP at 8 and 12WAIT had TQ of 6.5, significantly less than TE, PC, FL, and SI. At 16 and 20WAIT, EP had the lowest TQ of 5.6 and 5.5, respectively. This was due to yellowing turfgrass color, reduced density and visibly widening of leaf blades (data not shown). This supports Dernoeden (1984) and Diesburg and Christians (1989) who reported increased internode length and widening of leaf blades from EP applications.

Applications of TE, PC, FL, and SI had increased TQ over untreated at all rating dates besides 4WAIT (Table 24). These rates would be deemed safe for 'Diamond' zoysiagrass greens during peak season, but mowing factors need to be considered. Increased TQ from SI applications contrasts results from chapter 2 field applications, possibly due to more effective movement of chemistry off the leaves to the rootzone. PB provided increased density, but lower rates and intervals should be used for 'Diamond' zoysiagrass. EP is not a suitable PGR for 'Diamond' zoysiagrass greens due to decreased density, undesirable color and leaf blade widening.

Further research should investigate dose responses for desirable PGRs to determine thresholds for intervals and rates. Studies should also be repeated in peak growing season field settings with regular mowing.

Table 24. Turf quality response means of 'Diamond' zoysiagrass from 4 to 20 weeks after initial treatment (WIAT) from 30 January 2019 to 22 May 2019 in Clemson, South Carolina.

Treatment	4WAIT	8WAIT	12WAIT	16WAIT	20WAIT
			1 – 9		
Untreated	7.3bc	6.9b	6.8b	6.9b	6.8d
TE	7.6ab	7.8a	7.8a	7.9a	8.0a
PC	7.5abc	7.6a	7.6a	7.8a	7.9ab
FL	7.9a	7.8a	7.6a	7.8a	7.4bc
PB	7.4bc	6.8b	6.8b	7.0b	7.0cd
EP	7.1c	6.5b	6.5b	5.6c	5.5e
SI	7.4bc	7.8a	7.6a	7.9a	7.5abc
LSD	0.5	0.7	0.6	0.5	0.5
Significance	NS	***	***	***	***

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at the 0.05 probability level.
§ Turf quality rated from 1 – 9 where 9 = best turf and values < 7.0 are unacceptable.

Normalized Difference of Vegetative Index

NDVI had a significant treatment by date effect, p-value 0.0026, warranting focus on individual dates. No treatment by repetition effect was observed so results were combined.

An overall date effect across repetitions occurred as NDVI was increased at 4 and 8WAIT, but slowly declined to 20WAIT, similar to TQ (Table 25). At 16WAIT, SI increased NDVI by 5% over untreated. At 20 WAIT, TE and EP had 6 and 3% reductions from untreated. PC, FL and PB were statistically similar to untreated at all dates.

EP decreased both TQ and NDVI, illustrating a loose correlation between TQ and NDVI. In contrast, TE increased TQ but reduced NDVI. SI increased TQ and NDVI. Differences in density between treatments may have an effect on differences in NDVI.

Table 25. Normalized difference of vegetative index response means of ‘Diamond’ zoysiagrass over 20 weeks from 30 January 2019 to 22 May 2019 in Clemson, South Carolina.

Treatment	4WAIT	8WAIT	12WAIT	16WAIT	20WAIT
			0 – 0.999 (best)		
Untreated	0.731ab	0.748ab	0.707abc	0.708b	0.719ab
TE	0.736ab	0.737b	0.683c	0.701b	0.679c
PC	0.733ab	0.735b	0.689bc	0.710ab	0.697bc
FL	0.752a	0.759a	0.723ab	0.714ab	0.701bc
PB	0.727b	0.760a	0.713abc	0.690b	0.695bc
EP	0.717b	0.735b	0.714abc	0.684b	0.691c
SI	0.729b	0.760a	0.741a	0.741a	0.739a
LSD	0.021	0.021	0.038	0.032	0.027
Significance	NS	*	NS	*	*

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
* Significant at the 0.05 probability level.
‡ NS, nonsignificant at the 0.05 probability level.
§ NDVI values ranged from 0 – 0.999, where 0.999 = best possible color.

Seedhead Count

Seedhead count had a significant treatment by date effect, p-value <0.0001, warranting focus on individual dates. No treatment by repetition effect was observed so results were combined.

At 12WAIT, EP had a 115% increase in seedhead count from untreated, and was significantly greater than all treatments besides PC and FL (Table 26). At 16WAIT, SI reduced count by 93% from untreated. At 16WAIT, EP increased count by 336% from untreated. At 16WAIT, TE and PC decreased count by 46 and 43% from untreated, respectively. At 20WAIT, SI decreased count by 63% from untreated. At 20WAIT, TE had a 41% decrease from untreated, and was statistically similar to SI. At 20WAIT, PB and EP increased count by 31 and 67% from untreated, respectively. FL and PC were never significantly different from untreated.

Bunnell (2003) reported FL reduced seedhead production in perennial ryegrass and Kentucky bluegrass, but these findings were not repeated on ‘Diamond’ zoysiagrass. King et al. (2006) reported PB to decrease *Lolium temulentum* seedhead production, but the present study did not have similar results on ‘Diamond’ zoysiagrass. Multiple studies of EP applications to annual bluegrass provided seedhead reductions (Gelernter and Stowell, 2001; Haguewood et al., 2013; Kane and Miller; 2003; McCullough and Sidhu, 2014), but these results were not repeated in this study with ‘Diamond’ zoysiagrass. TE reductions in seedhead count were inconsistent with results from Chapter 2, possibly due to repeat applications. Qian et al. (1998) noted TE visually increased seedhead production in ‘Diamond’ zoysiagrass. No previous reports have observed TE or SI providing seedhead control on ‘Diamond’ zoysiagrass.

Further research should investigate effectiveness of TE and PC repeat applications to reduce seedhead production in field settings. Research should also investigate similar herbicides to SI to reduce seedhead production to prevent herbicide resistance by weeds.

Table 26. Seedhead count response means of ‘Diamond’ zoysiagrass from 4 to 20 weeks after initial treatment (WIAT) from 30 January 2019 to 22 May 2019 in Clemson, South Carolina.

Treatment	4WAIT	8WAIT	12WAIT	16WAIT	20WAIT
			count plot^{-1}		
Untreated	0a	0a	4.0bc	14.9bc	66.1c
TE	0a	0a	2.6bc	6.9cd	39.1de
PC	0a	0.4a	4.8abc	8.5cd	53.5cd
FL	0a	0.3a	5.6ab	19.3b	58.5c
PB	0a	0a	3.3bc	22.1b	86.9b
EP	0a	0a	8.6a	51.6a	110.6a
SI	0a	0a	0.6c	1.3d	24.5e
LSD	0	0.5	4.2	10.3	19.1
Significance	NS	NS	*	***	***

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level**.**
‡ NS, nonsignificant at the 0.05 probability level.
§ Seedheads were counter in each 10.8 cm plot, where lower counts are desirable.

Clipping Mass

Clipping mass had a significant treatment effect, p-value <0.0001. SI increased clipping weight by 37% from untreated (Table 27). FL, PB, PC and TE had reductions of 48, 49, 51 and 66% from untreated, respectively. This supports McCullough et al. (2006) and McCullough et al. (2005c) who reported EP to be less effective in reducing growth than other PGRs. These findings also support Kreuser (2015), Kreuser and Soldat (2011), and Beam (2004) who reported PC to provide similar growth suppression levels to TE. These findings also support Ries and Wert (1972) who reported increased yields with SI.

These results illustrate TE, PC and FL were able reduce clipping mass while increasing TQ, while PB decreased both clipping mass and TQ. These results also revealed the ineffectiveness of EP to reduce clipping mass. Further research should investigate a GDD model for *Z. matrella* putting greens similar to Reasor et al. (2018) and Kreuser et al. (2018) using TE and PC.

Table 27. Clipping mass response means of 'Diamond' zoysiagrass from 24 weeks after initial treatment (WIAT) from 19 June 2019 in Clemson, South Carolina.

Treatment	24WAIT
	g $plot^{-1}$
Untreated	2.94b
TE	0.99c
PC	1.43c
FL	1.54c
PB	1.49c
EP	2.78b
SI	4.03a
LSD	0.69
Significance	***

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
*** Significant at the 0.001 probability level**.**
‡ NS, nonsignificant at the 0.05 probability level.
§ Root mass was determined via loss by ignition of roots removed below thatch, where higher weights are desirable.

Root Mass

Root mass had no treatment effect, p-value 0.4113. McCullough et al. (2005a) and McCarty et al. (2011) reported FL to decrease root density, which was not repeated in this study. McCullough et al. (2006) and McCullough et al. (2005c) also reported EP to significantly decrease root mass and density. These results were not repeated in the present study. 'Diamond' zoysiagrass had a high tolerance to all tested PGRs in regards to rooting.

Table 28. Root mass response means of 'Diamond' zoysiagrass from 24 weeks after initial treatment (WIAT) from 19 June 2019 in Clemson, South Carolina.

Treatment	24WAIT
	g plot^{-1}
Untreated	1.18a
TE	1.10a
PC	0.99a
FL	0.91a
PB	0.85a
EP	1.13a
SI	0.90a
LSD	0.35
Significance	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (.10).
‡ NS, nonsignificant at the 0.1 probability level.
§ Clipping mass was determined via loss by ignition of clippings removed 3 cm above soil, where lower weights are desirable.

CHAPTER FOUR

'DIAMOND' ZOYSIAGRASS (*Zoysia matrella* (L.) Merr.) LATERAL RECOVERY USING REPEAT APPLICATIONS OF PLANT GROWTH REGULATORS

Introduction

'Diamond' zoysiagrass (*Zoysia matrella* (L.) Merr) has proven to be extremely slow to recover from lateral growth (Sladek et al., 2009). This is an undesirable trait for turfgrasses, as cultural practices and traffic damage will take longer to recover from surface damage such as ball marks, environmental stresses or traffic. 'Diamond' zoysiagrass putting greens should be core aerified at minimum once season^{-1} to reduce thatch development (McCarty, 2018). Lateral recovery from aerification is a nuisance to turfgrass managers and golfers, and factors in recovery time may include plant growth regulators (PGRs).

Materials and Methods

A study was conducted from January 2019 to October 2019 at Clemson University Greenhouse Complex in Clemson, SC with the objective to determine if PGRs applied repeatedly reduced lateral growth. 'Diamond' zoysiagrass plugs 10.8 cm diameter and 15 cm depth were harvested in October 2018 from a nursery green at Walker Golf Course, which was originally constructed in June 2014 from sod over a converted creeping bentgrass green with USGA soil mix originally constructed in 1995 (personal communication with Don Garrett, 2018). Plugs were established in 15 cm diameter and 15 cm depth pots with USGA greens mix of 85 sand:15 peatmoss by volume (USGA, 2018). Plants were maintained with 14 h photoperiod and a light intensity of 500 µmol m^{-2} s^{-1}, with day/night temperatures maintained near 28/19°C.

A 3.75 cm diameter and 5 cm depth core was removed from each plug and filled with USGA greens mix on 2 January 2019 (USGA, 2018). Treatments were applied using an enclosed spray chamber (DeVries Manufacturing, Hollandale, MN) on a 4 wk interval beginning 2 January 2019 and included trinexapac-ethyl (TE), paclobutrazol (PB), ethephon (EP), simazine (SI), flurprimidol (FL) and prohexadione calcium (PC) (Table 29). All treatments also included a non-ionic surfactant (NIS) (Harrell's SprayMAX, Lakeland, FL) at a 0.25% volume $volume^{-1}$ rate. Foliar fertilization using Grigg Gary's Green 18-3-4 (Brandt Consolidated Inc. Springfield, IL) at 4.9 g N m^{-2} was also included in each application including untreated control plots. Pots were left to dry for 4 h after treatment application to satisfy leaf and sheath absorbed PGRs, then irrigated with 1.27 cm water to satisfy requirements for root absorbed PGRs. Pots were mowed weekly at 5 mm using shears and watered as needed to prevent wilt.

Table 29. Treatments and rates used in 'Diamond' zoysiagrass lateral recovery using repeat applications of plant growth regulators study.

Treatment[a]	Standard Rate (lb a.i. $acre^{-1}$)	Metric Rate (g a.i. ha^{-1})
Untreated	–	–
TE (Primo Maxx 1MEC)	0.0366	41
PB (Trimmit 2SC)	0.0598	67
EP (Proxy 2L)	3.0816	3,454
SI (Princep 4L)	0.8922	1,000
PC (Anuew 27.5WP)	0.1035	116
FL (Cutless MEC 1.3L)	0.0834	93.5

[a] All treatments also included a NIS at a 0.25% volume $volume^{-1}$ rate, and liquid fertilizer at 4.9 g N m^{-2}.

Statistical Design

Statistical design was a randomized complete block with two repetitions and four blocks and repeated observations at different rating dates. Data were subjected to an overall ANOVA (Table 30) to determine treatment effects across all rating dates and any treatment by rating date interactions. Since treatment by rating date interactions were significant, rating data were subjected to ANOVA at each rating date to assess treatment effects. All measurements besides root mass were further studied using Fisher's protected LSD at a significance level of 0.05. Data were analyzed using JMP Pro 14.1 software (SAS Institute Inc.; Cary, NC, USA).

Table 30. Analysis of variance (ANOVA) table of a randomized complete block design for lateral recovery means of 'Diamond' zoysiagrass lateral recovery using repeat applications of plant growth regulators study.

Source of Variation	df[a]	Lateral Recovery Rate
Treatment	6	***
Block (Repetition) Error	3	**
Treatment x Block (Repetition) Error	18	***
Repetition	1	NS
Treatment x Repetition	6	NS
Date	9	***
Treatment x Date	54	***
Error	441	
Corrected Total	538	

[a] Degrees of freedom
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at the 0.05 probability level.

Measurements

Lateral recovery was rated by percent lateral recovery every 4 wk beginning 30 January 2019 using a grid intersect count from a circular wire grid 3.81 cm diameter and 210 countable squares. Percent lateral recovery was determined using the formula

$$\%\ Lateral\ Recovery = 'hits'\ per\ plot \div 210$$

where when green leaf tissue is present in a square it is counted a 'hit' and sand is not (Atkinson, 2010).

Results and Discussion

Lateral Recovery

Lateral recovery had significant date by treatment effect, p-value <0.0001, warranting focus on individual dates. No repetition effect was detected, so data were combined. Lateral recovery from initial treatment to 4WAIT was more rapid than any 4 wk period during this study, all treatments having recovered at least 40% by this date (Table 31). This is due to leaves utilizing horizontal growth to capture light more efficiently, and subsequently intersecting outermost grid squares. However, stolons and rhizomes were slower to develop and fully recover in all treatments, illustrated by first treatments to reach 95% recovery taking 16WAIT and 100% recovery taking 24WAIT.

At 16WAIT, EP was the first treatment to reach 95% recovery (Table 31). This supports McCullough et al. (2005c) who reported EP was not effective in suppressing growth. At 20WAIT, untreated and SI reached 95% recovery. This supports Fry et al. (1986) who reported SI increased tillering. At 24WAIT, PB reached 95% recovery, the most rapid lateral recovery rate of gibberellic acid inhibiting compounds. At 28WAIT, PC, TE and FL reached 95% recovery. At 16 and 20WAIT, PC and TE were significantly more recovered than FL, and at 24WAIT, PC was significantly more recovered than FL. These findings support multiple studies that found gibberellic acid inhibiting PGRs decreased internode length and growth rates.

This study displayed an undesirable trait of 'Diamond' zoysiagrass. Low nitrogen rates likely exacerbated slow recovery time, but gibberellic acid inhibiting compounds, FL, PB, PC, and TE, all significantly slowed lateral recovery time from untreated at multiple dates. These results suggest field managers cease using class A and B PGR during core aerification or recovery situations. Future research should investigate if nitrogen fertility and biostimulants hasten lateral recovery.

Table 31. Percentage lateral recovery response means of ‘Diamond’ zoysiagrass from 4 to 40 weeks after initial treatment (WIAT) from 30 January 2019 to 9 October 2019 in Clemson, South Carolina.

	WAIT									
Treatment	4	8	12	16	20	24	28	32	36	40
					0 – 100% Lateral Recovery					
Untreated	48.7a	58.2ab	82.0a	91.6a	97.0a	98.8ab	100a	100a	100a	100a
TE	41.7bc	49.2c	67.6c	77.3b	85.2c	88.3de	95.4c	97.7c	99.2b	100a
PC	40.1c	48.8c	68.2c	80.0b	86.9bc	92.0cd	96.8bc	98.3bc	99.3b	100a
FL	47.4ab	54.4bc	65.1c	71.8c	80.5d	87.0e	95.2c	98.4bc	99.6ab	100a
PB	48.5ab	58.9ab	74.3b	80.5b	91.4b	94.7bc	98.5ab	99.3ab	100a	100a
EP	47.7ab	61.5a	84.0a	96.0a	99.8a	100a	100a	100a	100a	100a
SI	49.7a	63.9a	84.5a	91.6a	98.2a	99.6a	100a	100a	100a	100a
LSD	6.9	7.0	6.1	5.0	4.5	4.4	1.9	1.3	0.6	0
Significance	*	***	***	***	***	***	***	**	*	NS

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
‡ NS, nonsignificant at the 0.05 probability level.
§ Percent lateral recovery was determined by counting grid intersections, and higher values are desirable.

CONCLUSION

'Diamond' zoysiagrass has many attractive qualities for turfgrass managers looking for an alternative to ultradwarf bermudagrass or creeping bentgrass putting greens. High shade tolerance compared to bermudagrass and greater heat and disease tolerance compared to bentgrass can reduce inputs required to maintain acceptable turf quality. However, 'Diamond' zoysiagrass cannot provide exceptional putting distances, but smoothness of roll can be compromised by seedhead presence (data not shown).

Treating 'Diamond' zoysiagrass with SI can provide effective and nearly season-long control, proven by success in both greenhouse and field trials. Treating with EP and PB resulted in increased seedhead production in greenhouse trials but was not replicated in the field. No treatment improved TQ or NDVI over untreated in the field, but greenhouse results had significant improvements in density with TE, PC, FL and PB applications. These results warrant research into regular applications in field settings, especially during summer months. Lateral recovery was decreased from treatment with TE, PC, FL and PB. These results support ceasing PGR use during aerification to reduce lateral recovery time.

FIGURES

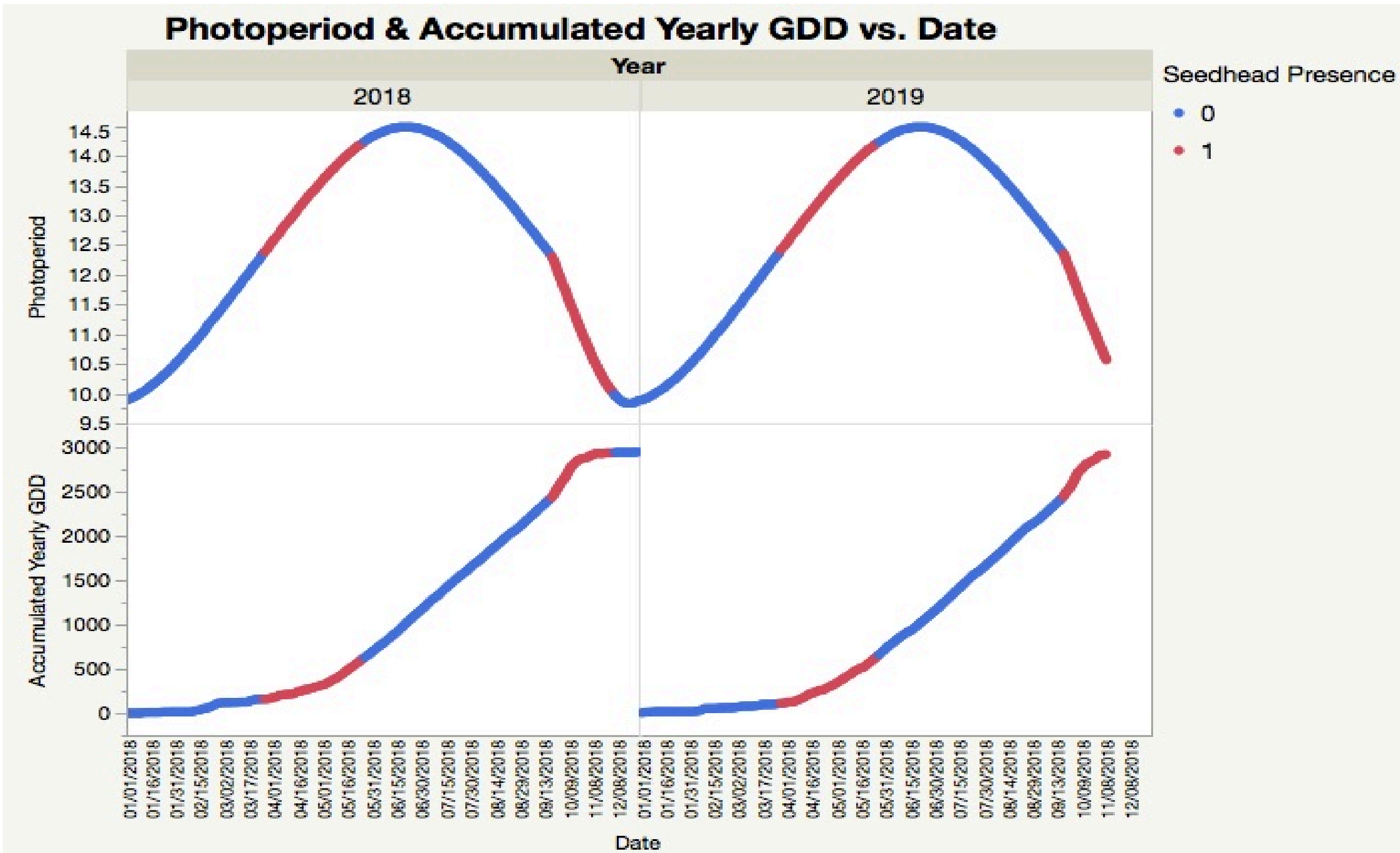

Figure 1. ‘Diamond’ zoysiagrass’ seedhead presence (red) was recorded throughout Chapter 1 field study. This graph illustrates the ability of both photoperiod and GDD to predict accurately initial seedhead emergence dates. However, as photoperiod is consistent year to year while GDD is not, these results need further evidence to separate the better predictor.

Figure 2. During fall seasons severe seedhead pressure on ‘Diamond’ zoysiagrass putting greens may impact quality of cut or ball roll smoothness and distances. This area had not been mowed in 2 weeks to observe ‘Diamond’ zoysiagrass at collar height. This reduction in mowing allowed seedheads to flourish and mature, showing mowing is a cultural practice somewhat sufficient in reducing seedhead pressure..

Figure 3. ‘Diamond’ zoysiagrass’ seedhead presence impacts ball roll smoothness and distance. These seedheads typically protrude from the canopy, creating inconsistencies in height and texture that a seedhead-free surface would lack.

Figure 4. Initial seedhead emergence in ‘Diamond’ zoysiagrass results young light colored seedheads, while mature seedheads are a purple-red color

Figure 5. On 4 November 2019 (56DAT) treatments containing simazine (dark green) compared to all other treatments with abundant light colored seedheads. Seedhead control for this duration is very unusual and desirable for turfgrass managers.

Figure 6. A seedhead count untreated control sample from 4 November 2019 (56DAT).

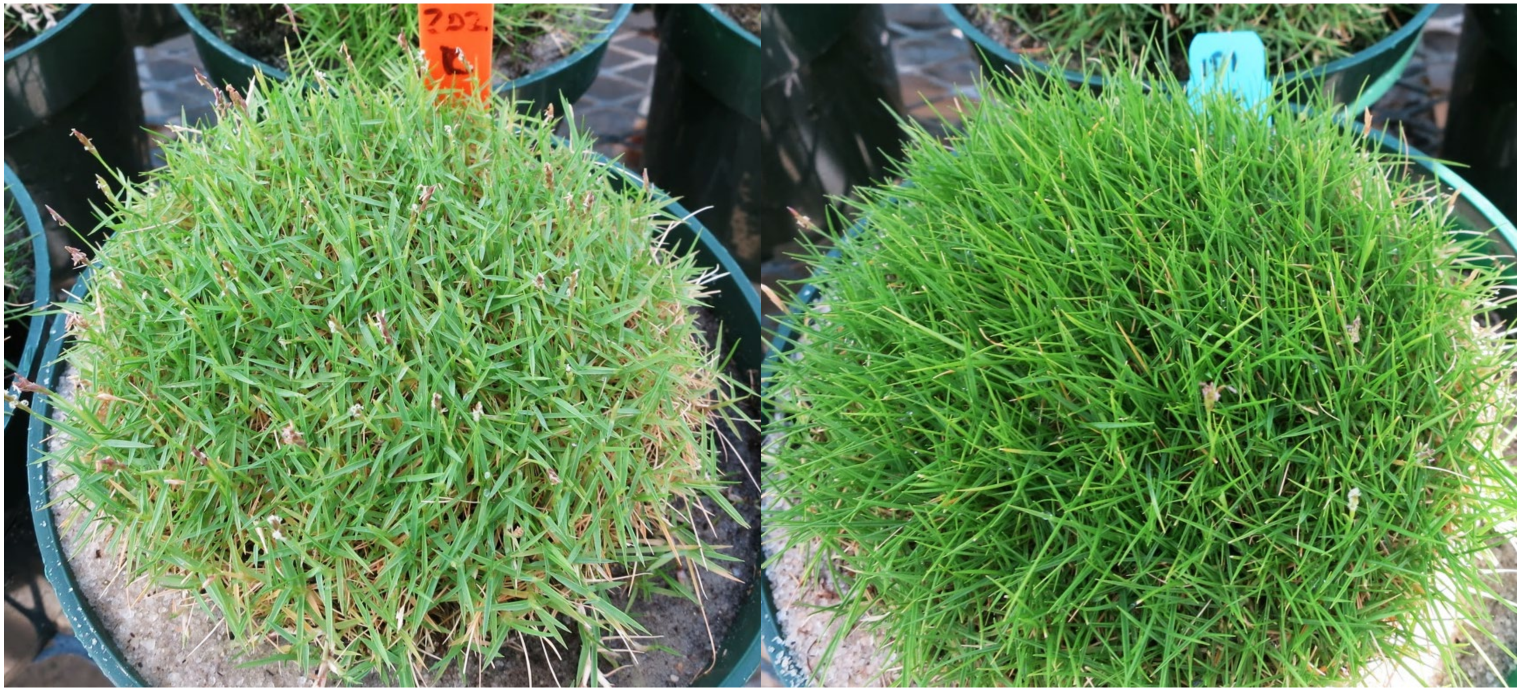

Figure 7. Ethephon treated 'Diamond' zoysiagrass (left) compared to untreated (right) after 4 sequential treatments from Chapter 3. Ethephon treatments caused increased seedhead production, visibly wider leaf blades, and undesirable color.

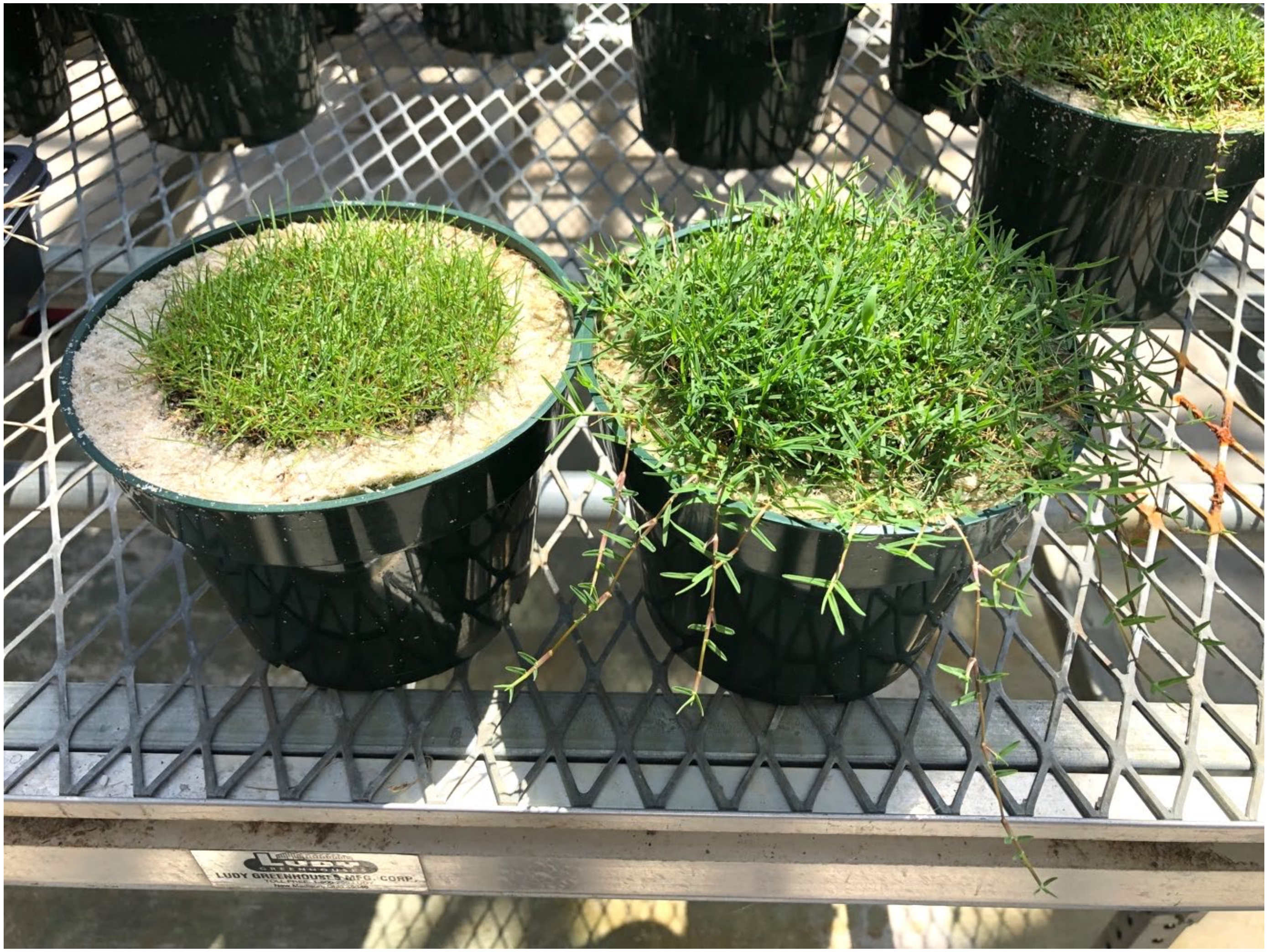

Figure 8. ‘Diamond’ zoysiagrass (left) and ‘Tifeagle’ bermudagrass (right) exhibit lateral growth differences after a similar duration in greenhouse.

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